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Tachas**(10) **Patent No.: US 9,821,034 B2**(45) **Date of Patent: Nov. 21, 2017**(54) **COMBINATION THERAPY**(71) Applicant: **Antisense Therapeutics Ltd**, Toorak,
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See application file for complete search history.

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Primary Examiner — Jane Zara(74) *Attorney, Agent, or Firm* — Knobbe, Martens,
Olson & Bear, LLP(57) **ABSTRACT**The present disclosure relates to a method for treatment or
prevention of diseases have an increased level of insulin-like
growth factor I (IGF-I). The method comprises administra-
tion of a growth hormone (GH) variant having antagonistic
activity in combination with an oligonucleotide targeted to
growth hormone receptor (GHR) to a subject in need.**21 Claims, No Drawings**

1

COMBINATION THERAPY**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a divisional of U.S. patent application Ser. No. 14/376390, filed Aug. 1, 2014, which is a U.S. National Phase under 35 U.S.C. §371 of International Application No. PCT/AU2013/000095, filed Feb. 4, 2013, which claims priority to the U.S. Patent Application No. 61/594532, filed Feb. 3, 2012, each of which is hereby incorporated by reference in its entirety into this application.

SEQUENCE LISTING

A Sequence Listing is provided herewith as a text file, FBRIC67 003D1.txt created on Jan. 26, 2016 and having a size of 425 KB. The contents of the text file are incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

The present disclosure relates to a method for treatment or prevention of diseases caused by and/or associated with an increased level of insulin-like growth factor I (IGF-I) that relies on administration of a growth hormone (GH) variant having antagonistic activity in combination with an antisense oligonucleotide targeted to the growth hormone receptor (GHR).

BACKGROUND OF THE INVENTION

Growth hormone (GH), released by the pituitary, is a member of a cascade of hormones that regulate growth of the body and its organs. Secretion of GH into the bloodstream is followed by binding to growth hormone receptor (GHR) on many cell and organ types. Growth hormone signaling is mediated by this interaction. Growth hormone signaling causes the production of another hormone, insulin-like growth factor I (IGF-I), which is produced in the liver, adipose tissue, kidney and other organs and secreted into the bloodstream. About 75% of serum IGF-I is produced in the liver in response to GH stimulation. Many disorders are caused by and/or associated with increased GH levels and/or increased IGF-I levels in plasma and/or tissues including acromegaly, gigantism, retinopathy, macular degeneration, nephropathy, diabetes and cancers. The role of GH and IGF-I in these and other disorders is well recognized. The role of IGF-I in mediating many GH effects is well recognized and this interrelationship is referred to as the GH/IGF-I axis. In a normal feedback loop, IGF-I also causes the production of GH by the pituitary to be reduced. There is a need for treatments that reduce IGF-I levels in a subject, for example, more effectively, safely, conveniently and/or at reduced cost.

Somavert, a GH variant having antagonistic activity is approved in the treatment of acromegaly for its ability to reduce serum IGF-I levels in a patient. For a review of current practices for the treatment of acromegaly see Guistina et al., 2011. Briefly, in acromegaly, surgery is first used in treatment to debulk the tumor and reduce the pituitary tumor's GH secretion and reduce production of IGF-I in the serum. Medicinal treatments are also used to reduce serum IGF-I and in some cases also reduce GH release. All treatments use a medicinal monotherapy or a combination therapy where the combination is directed to two different biological targets. First line medicinal treatment is with a

2

somatostatin (SST) agonist, and this treatment is started initially at low doses and escalated to doses that reduce GH and normalize the patients serum IGF-I. When an SST agonist treatment fails, a dopamine agonist is used in combination with a SST agonist, or Somavert is used in combination with a SST agonist or Somavert is used as a monotherapy. Somavert doses typically start with a loading dose of 40 mg on the first day, and 10 mg daily doses, and these daily doses are escalated until normalization of serum IGF-I, with up to 30 mg daily doses approved. There are failures with the highest approved daily doses of Somavert, and clinicians have escalated beyond approved doses. Somavert is however, prohibitively expensive, requires inconvenient daily (once or twice daily) injection, is a lyophilized powder and needs reconstitution, has safety issues causing injection site reactions and an increase in liver enzymes, and produces undesirable increases in GH. Thus, clinicians typically do not seek to use Somavert in different combination therapies to those outlined above. In patients where first line therapy using SST has failed or in patients where Somavert monotherapy or combination with SST has failed, clinicians are seeking new more effective monotherapies. Combination therapies using, for example, Somavert are only considered with a drug directed to a different biological target and typically an SST agonist, as SST agonists have potential to reduce tumor size.

SUMMARY OF THE INVENTION

The present inventors have now made the surprising finding that a growth hormone (GH) variant having antagonistic activity and an antisense oligonucleotide targeted to GHR act synergistically to reduce insulin-like growth factor I (IGF-I) levels in a subject. In other words, the combined administration of the GH variant and the oligonucleotide to GHR exhibits greater than additive effect. This is surprising, particularly considering that there are no expected synergies with using drugs to the same target, and one can just escalate the dose of the drug to the target. Accordingly, the present disclosure provides a method for treatment or prevention of a disease caused by and/or associated with an increased level of IGF-I, the method comprising administering to a subject in need thereof, a GH variant having GH antagonistic activity in combination with an oligonucleotide 8 to 80 nucleobases in length targeted to a nucleic acid encoding GHR so as to inhibit expression of the GHR, thereby reducing the level of IGF-I in the subject. In one embodiment, the level of serum/plasma IGF-I is reduced.

In one embodiment, the method further comprises identifying a subject in need of a reduction in said subject's GHR and/or IGF-I levels, for example serum/plasma IGF-I levels.

In one embodiment, the disease is acromegaly, diabetic retinopathy, diabetic nephropathy, or an IGF-I positive and/or IGF-I and/or GH responsive cancer such as prostate, myeloma, lung, breast, or colon cancer.

In one embodiment, the GH variant is a human GH variant in which amino acid Gly120 is deleted or substituted with an amino acid, for example, Arg, Trp, Pro, Lys or Leu. In one embodiment, the Gly120 is substituted with Lys.

In a further embodiment, the human GH variant comprises the following set of amino acid substitutions: H18D, H21N, R167N, K168A, D171S, K172R, E174S, I179T.

In one embodiment, the nucleic acid encodes human GHR. The nucleic acid may have a nucleotide sequence as shown in SEQ ID NO:4 or SEQ ID NO:5.

In one embodiment, the oligonucleotide is from 12 to 50 nucleobases in length. In another embodiment, the oligonucleotide is from 15 to 30 nucleobases in length.

In one embodiment, the oligonucleotide is a DNA oligonucleotide. In another embodiment, the oligonucleotide is a RNA oligonucleotide, for example, a short interfering RNA (siRNA). In another embodiment, the oligonucleotide is a chimeric oligonucleotide.

In one embodiment, the oligonucleotide has at least 70% complementarity with the nucleic acid encoding GHR. In another embodiment, the oligonucleotide has at least 80% complementarity with the nucleic acid encoding GHR. In another embodiment, the oligonucleotide has at least 90% complementarity with the nucleic acid encoding GHR. In another embodiment, the oligonucleotide has at least 95% complementarity, for example, 96%, 97%, 98%, or 99% complementarity with the nucleic acid encoding GHR.

In one embodiment, the oligonucleotide comprises at least an 8 consecutive nucleobase portion of SEQ ID NO: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, or 81.

In another embodiment, the oligonucleotide consists of the nucleobase sequence of SEQ ID NOs: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, or 81.

In one embodiment, the oligonucleotide consists of the nucleobase sequence of SEQ ID NO:6.

In one embodiment, the oligonucleotide specifically hybridises with a region encoding GHR, wherein the region comprises a translation initiation codon, a termination codon, a coding region, a 5' untranslated region, a 3' untranslated region, an intron:exon junction or an exon:intron junction. In one embodiment, the region comprises at least an 8 consecutive nucleobase portion of a sequence selected from SEQ ID NOs: 84-154.

In one embodiment, the oligonucleotide comprises at least an 8 consecutive nucleobase portion complementary to a region of SEQ ID NO:4 selected from the group consisting of nucleotides 260-339, 332-351 and 344-423 of SEQ ID NO:4.

In one embodiment, the oligonucleotide inhibits the expression of GHR and/or growth hormone binding protein (GHBP) by at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, or 45%.

In one embodiment, the oligonucleotide comprises at least one modified internucleoside linkage, sugar moiety, or nucleobase. The oligonucleotide may, for example, comprise at least one 2'-O-methoxyethyl sugar moiety and/or at least one phosphorothioate internucleoside linkage and/or at least one 5-methylcytosine.

In one embodiment, the oligonucleotide consists of 20 linked nucleosides, wherein the oligonucleotide consists of a nucleobase of SEQ ID NO:6; and wherein the oligonucleotide consists of a ten deoxynucleotide region flanked on both the 5' end and the 3' end of said ten deoxynucleotide region with five 2'-O-(2-methoxyethyl) nucleotides, and wherein each internucleoside linkage in the oligonucleotide is a phosphorothioate linkage, and wherein each cytosine in said oligonucleotide is a 5-methylcytosine.

The present disclosure also provides a method of reducing the level of IGF-I in a subject, the method comprising administering a GH variant having GH antagonistic activity in combination with an oligonucleotide 8 to 80 nucleobases in length targeted to a nucleic acid encoding GHR so as to inhibit expression of the GHR, thereby reducing the level of IGF-I in the subject. In one embodiment, the level of serum/plasma IGF-I is reduced.

In one embodiment, the method further comprises identifying a subject in need of a reduction in said subject's GHR and/or IGF-I levels, for example serum/plasma IGF-I levels.

The GH variant, the GHR and the oligonucleotide may be further characterized by any one of the above features.

The present disclosure also provides for use of a GH variant and an oligonucleotide 8 to 80 nucleobases in length targeted to a nucleic acid encoding GHR in the manufacture of a medicament for the treatment or prevention of a disease caused by and/or associated with an increased level of IGF-I. In one embodiment, the level of serum/plasma IGF-I is reduced.

In one embodiment, the disease is acromegaly, diabetic retinopathy, diabetic nephropathy, or an IGF-I positive cancer such as prostate, myeloma, lung, breast, or colon cancer.

The GH variant, the GHR and the oligonucleotide may be further characterized by any one of the above features.

The present disclosure also provides for use of a GH variant and an oligonucleotide 8 to 80 nucleobases in length targeted to a nucleic acid encoding GHR in the manufacture of a medicament for reducing the level of IGF-I in a subject.

The GH variant, the GHR and the oligonucleotide may be further characterized by any one of the above features.

The present disclosure also provides for a composition comprising a GH variant and an oligonucleotide 8 to 80 nucleobases in length targeted to a nucleic acid encoding GHR for the treatment or prevention of a disease caused by and/or associated with an increased level of IGF-I.

The GH variant, the GHR and the oligonucleotide may be further characterized by any one of the above features.

The present disclosure also provides for a composition comprising a GH variant and an oligonucleotide 8 to 80 nucleobases in length targeted to a nucleic acid encoding GHR for reducing the level of IGF-I in a subject.

The GH variant, the GHR and the oligonucleotide may be further characterized by any one of the above features.

Any embodiment herein shall be taken to apply mutatis mutandis to any other embodiment unless specifically stated otherwise.

The invention is hereinafter described by way of the following non-limiting Examples and with reference to the accompanying Figures.

KEY TO THE SEQUENCE LISTING

SEQ ID NO:1 Wild-type human growth hormone (hGH) nucleotide sequence
 SEQ ID NO:2 Wild-type hGH polypeptide sequence
 SEQ ID NO:3 Somavert polypeptide sequence
 SEQ ID NO:4 Human growth hormone receptor (hGHR) cDNA sequence
 SEQ ID NO:5 hGHR gene sequence
 SEQ ID NO:6-83 Oligonucleotides targeted to hGHR
 SEQ ID NO:84-154 Target sequences of hGHR

DETAILED DESCRIPTION OF THE
INVENTION

General Techniques and Selected Definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in antisense technology, recombinant technology, cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley and Sons (1984), J. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory Press (1989), T. A. Brown (editor), *Essential Molecular Biology: A Practical Approach*, Volumes 1 and 2, IRL Press (1991), D. M. Glover and B. D. Hames (editors), *DNA Cloning: A Practical Approach*, Volumes 1-4, IRL Press (1995 and 1996), F. M. Ausubel et al. (editors), *Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), E. Harlow and D. Lane (editors), *Antibodies: A Laboratory Manual*, Cold Spring Harbour Laboratory (1988), and J. E. Coligan et al. (editors), *Current Protocols in Immunology*, John Wiley and Sons (including all updates until present).

The term “and/or”, for example, “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein, “about” or “approximately” shall generally mean within 20%, more preferably within 10%, and even more preferably within 5%, of a given value or range.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, group of steps or group of compositions of matter.

Treatment and Prevention of IGF-I Positive Diseases

The present invention provides methods useful in the prevention and/or treatment of a disease, disorder, or condition caused by and/or associated with an increased level of insulin-like growth factor I (IGF-I). As used herein, the term “treatment” refers to administering a pharmaceutical composition to effect an alteration or improvement of a disease, disorder, or condition. As used herein, the term “prevention” refers to administering a pharmaceutical composition to stop or hinder the development of at least one symptom of a disease, disorder, or condition. The subject targeted for treatment is a mammal, preferably a human. As used herein, “an increased level of insulin-like growth factor I (IGF-I)” includes a level above the normal range or in the normal range, for example, at the high end of the normal range, adjusted for age and sex.

The methods involve administration of a growth hormone (GH) variant having GH antagonistic activity and an oligonucleotide targeted to growth hormone receptor (GHR) to a subject. The “subject” can be any mammal, preferably a human. Although not wishing to be limited to the theory, the oligonucleotide acts to inhibit GHR expression in said subject, whilst the GH variant acts to prevent GH binding to the GHR, thereby reducing the level of IGF-I (which is produced in response to GH signalling) in the subject. Insulin-like growth factor I is a ubiquitous polypeptide important in proliferation, having potent mitogenic effects on a broad range of cells, and important in cell survival, regulating apoptosis on a broad range of cells.

Although not wishing to be limited to theory, the antisense oligonucleotide inhibits GHR expression at the RNA level whilst the GH variant may increase GHR RNA by feedback inhibition. In one embodiment, the oligonucleotide also acts to reduce growth hormone binding protein (GHRP) expression. The GHRP is the soluble extracellular portion of the GH receptor, derived by alternative mRNA splicing of the mRNA transcript (in for example, mice and rats) or proteolytic cleavage of the GHR (in for example, humans, cows and pigs).

In one embodiment, the treatment reduces or prevents occurrence of one or more symptoms of acromegaly, for example, reducing the increased serum IGF-I levels in acromegaly to normal levels, or reducing soft tissue swelling, enlargement of internal organs, extremities like overgrowth of the jaw, enlargement of hands and feet, deepening of the voice, thickening of skin, offensive body odor, articular cartilage problems, hyperphosphatemia, peripheral neuropathies, higher blood pressure, diabetes, heart disease, and cancer.

In another embodiment, the treatment reduces or prevents occurrence of one or more symptoms of retinopathy, for example, reducing new blood vessel formation and/or edema, blurred, double, or distorted vision, or difficulty reading, floaters or spots in vision, loss of vision or a shadow or veil across field of vision, pain, pressure, or constant redness of the eye.

In another embodiment, the treatment reduces or prevents occurrence of one or more symptoms of diabetic nephropathy, for example glomerula filtration, microalbuminuria, proteinuria, renal damage, swelling in the legs, nausea and vomiting, malaise, fatigue, headache, itching, frequent hiccups, unintended weight loss, swelling of the face, unintended weight gain due to fluid buildup, and high blood pressure.

In another embodiment, the treatment reduces the size and/or growth of a tumor or cancer (such as prostate, myeloma, lung, breast, or colon cancer) and/or delays progression of the tumor or cancer (such as prostate cancer) from androgen responsive/dependent to androgen unresponsive/independent. Tumor or cancer size and/or growth may be reduced, for example, by reducing the proliferation rate of the tumor/cancer cells, increasing the apoptotic rate of the tumor/cancer cells, modulating tumor/cancer cell signaling, chemosensitization, and/or inhibiting adhesion, anchorage, metastasis of the tumor/cancer cells and/or transformation of cells, for example, prostate cells. The treatment may, for example, reduce IGF-I levels at the high end of the normal range to lower levels, and/or from the top quartile to the 2nd quartile, 3rd or 4th quartile, and/or from the 2nd quartile to the 3rd or 4th quartile as adjusted for age and sex. The treatment may reduce endocrine, autocrine or paracrine levels of IGF-I as antisense oligonucleotides and the GH variant may work in the tissues.

Growth Hormone Variant Having Growth Hormone Antagonistic Activity

The methods of the present disclosure rely on the use of growth hormone (GH) variants having GH antagonistic activity. In one example, the GH variant is a peptide or protein having a similarity in sequence and/or secondary structure to a vertebrate GH, including but not limited to, mammalian growth hormones such as human and bovine growth hormones.

GH is synthesized and secreted by the somatotroph cells of the anterior pituitary gland. The GH gene consists of 5 exons and 4 introns encoding a 217-amino acid precursor protein. The amino-terminal signal peptide is removed by proteolysis, yielding the mature single chain 191-amino acid polypeptide, with a molecular mass of 22 kDa. The 3-dimensional structure of human (h) GH and of GH from other mammalian species was established by X-ray crystallography (Ultsch et al., 1991; Ultsch et al., 1993; de Vos et al., 1992). The protein consists of four α -helices, with 20-30 amino acid residues bound together by stretches of non-helical chains which are packed together in an antiparallel bundle (Ultsch et al., 1994). It has been demonstrated that the 4 alpha helix bundle GH possesses two non-identical binding surfaces, but binds to similar receptor binding sites in an ordered sequence, with the initial binding site possessing a higher affinity (Site 1) (WO 92/21029). Site 2 binding is stabilized by a further inter-receptor interaction involving the "dimerization domain" in the lower of the two cytokine homology modules (Cunningham et al., 1991; de Vos et al., 1992; Chen et al., 1997).

In one embodiment, the GH variant comprises an alpha helix having an amino acid sequence homology of at least about 50% with the third alpha helix of a vertebrate GH. The other alpha helices of the wild-type GH may be omitted if this can be done without loss of GH antagonist activity. The use of the term "antagonist" is in a functional sense and is not intended to limit the disclosure to compounds having a particular mechanism of action. Suitable GH variants are described in U.S. Pat. Nos. 5,350,836 and 5,849,535.

Variant GH sequence notation defines the actual amino acid substitutions in the GH variant. For a variant, substitutions are indicated by a letter representing the wild-type residue (in single-letter code), a number indicating the amino acid position in the wild-type sequence, and a second letter indicating the substituted amino acid residue. For example, G120K indicates a mutation in which glycine at position 120 is substituted with lysine. Multiple mutants are indicated by a series of single mutants separated by commas.

In one embodiment, the growth hormone variant is an hGH variant. The DNA (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of wild-type hGH have been reported (Goeddel et al., 1979; Gray et al., 1985).

In one embodiment, the hGH variant comprises a mutation at amino acid Gly120. Gly120 may be deleted or substituted with an amino acid. In one example, the amino acid is selected from the group consisting of Arg, Trp, Pro, Lys and Leu. In a preferred example, the amino acid is Lys. This mutation disrupts Site 2 binding. An hGH variant comprising this mutation acts as an hGH antagonist.

In a further embodiment, the hGH variant comprises the following set of amino acid substitutions: H18D, H21N, R167N, K168A, D171S, K172R, E174S, I179T. These substitutions increase binding affinity for the hGH receptor at Site 1. An hGH variant including this set of amino acid substitutions acts as an hGH agonist in the absence of an additional modification that disrupts binding to the hGHR at Site 2.

In one embodiment, the hGH variant comprises a G120 amino acid deletion or substitution and amino acid substitutions H18D, H21N, R167N, K168A, D171S, K172R, E174S, I179T. In one embodiment, the hGH variant includes the following set of amino acid substitutions: H18D, H21N, G120K, R167N, K168A, D171S, K172R, E174S, I179T. In one embodiment, the hGH variant is Somavert® (Pegvisomant for injection) (SEQ ID NO:3) which is a protein with 191 amino acid residues to which several polyethylene glycol (PEG) polymers are covalently bound (van der Lely et al., 2001).

Mutagenesis

The DNA sequence encoding GH can be mutated at one or more selected codons. A mutation is defined as a substitution, deletion, or insertion of one or more nucleotides in the DNA encoding the GH that results in a change in the amino acid sequence of the GH as compared with the wild-type sequence of the GH. Preferably, at least one amino acid is substituted with any other amino acid in one or more regions of the protein.

Site-specific mutagenesis (Carter et al., 1986; Zoller et al., 1987), cassette mutagenesis (Wells et al., 1985), restriction selection mutagenesis (Wells et al., 1986), or other known techniques can be performed on GH DNA to produce the variant DNA that encodes for the changes in the amino acid sequence.

Oligonucleotide-mediated mutagenesis is the preferred method for preparing substitution, deletion, or insertion variants of GH. The technique is well known in the art as described by Zoller et al., 1987. Briefly, an oligonucleotide encoding the desired mutation is hybridized to a DNA template which comprises the single-stranded form of the wild-type DNA sequence for GH. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template, and thus incorporates the oligonucleotide primer and codes for the selected alteration in the GH DNA.

Generally, oligonucleotides of at least 25 nucleotides in length are used. Although smaller oligonucleotides can be employed, an optimal oligonucleotide has 12 to 15 nucleotides that are complementary to the template on either side of the nucleotide(s) coding for the mutation(s). This ensures that the oligonucleotide hybridizes properly to the single-stranded DNA template molecule. The oligonucleotides are readily synthesized using techniques known in the art such as that described by Crea et al., 1978.

The DNA template can only be generated by those vectors that are either derived from bacteriophage M13 vectors (the commercially available M13mp18 and M13mp19 vectors are suitable), or those vectors that contain a single-stranded phage origin of replication as described by Vieira and Messing, 1987. Thus, the DNA that is to be mutated must be inserted into one of these vectors in order to generate single-stranded template. Production of the single-stranded template is described in sections 4.21-4.41 of Sambrook et al., supra.

To alter the wild-type DNA sequence, the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form, and the other strand (the original template) encodes the wild-type GH. This heteroduplex molecule is then transformed into a suitable host cell, usually a prokaryote such as *Escherichia coli* JM101. After

the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer radiolabeled with 32-phosphate to identify the bacterial colonies that contain the mutated DNA.

The method described immediately above can be modified such that a homoduplex molecule is created wherein both strands of the DNA contain the mutation(s). The modifications are as follows: The single-stranded oligonucleotide is annealed to the single-stranded DNA template as described above. A mixture of three deoxyribonucleotides, deoxyriboadenosine (dATP), deoxyriboguanosine (dGTP), and deoxyribothymidine (dTTP) is combined with a modified thio-deoxyribocytosine called dCTP-(aS). This mixture is added to the template-oligonucleotide complex. Upon addition of DNA polymerase to this mixture, a strand of DNA identical to the template except for the mutated base(s) is generated. In addition, this new strand of DNA contains dCTP-(AS) instead of dCTP, which serves to protect it from restriction endonuclease digestion. After the template strand of the double-stranded heteroduplex is nicked with an appropriate restriction enzyme, the template strand can be digested with ExoIII nuclease or another appropriate nuclease past the region that contains the site(s) to be mutagenized. The reaction is then stopped to leave a molecule that is only partially single-stranded. A complete double-stranded DNA homoduplex is then formed using DNA polymerase in the presence of all four deoxyribonucleotide triphosphates, ATP, and DNA ligase. This homoduplex molecule can then be transformed into a suitable host cell such as *E. coli* JM101, as described above.

Mutants with more than one amino acids to be substituted can be generated in one of several ways. If the amino acids are located close together in the polypeptide chain, they can be mutated simultaneously using one oligonucleotide that codes for all of the desired amino acid substitutions. If however, the amino acids are located some distance from each other (separated by more than about ten amino acids), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods can be employed.

In the first method, a separate oligonucleotide is generated for each amino acid to be substituted. The oligonucleotides are then annealed to the single-stranded template DNA simultaneously, and the second strand of DNA that is synthesized from the template encodes all of the desired amino acid substitutions. The alternative method involves two or more rounds of mutagenesis to produce the desired mutant. The first round is as described for the single mutants: wild-type DNA is used for the template, an oligonucleotide encoding the first desired amino acid substitution(s) is annealed to this template, and the heteroduplex DNA molecule is then generated. The second round of mutagenesis utilizes the mutated DNA produced in the first round of mutagenesis as the template. Thus, this template already contains one or more mutations. The oligonucleotide encoding the additional desired amino acid substitution(s) is then annealed to this template, and the resulting strand of DNA now encodes mutations from both the first and second rounds of mutagenesis. This resultant DNA can be used as a template in a third round of mutagenesis, and so on.

Cassette mutagenesis is also a preferred method for preparing substitution, deletion, and insertion variants of DNA encoding GH. The method is based on that described by Wells et al., 1985. The starting material is a plasmid (or other vector) comprising the GH DNA to be mutated. The nucleotide(s) in the GH DNA to be mutated are identified, optimally, there is a unique restriction endonuclease site on

each side of the identified mutation site(s); however, this is not a requirement. If no such restriction sites exist, they can be generated using the above-described oligonucleotide-mediated mutagenesis method to introduce them at appropriate locations in the GH DNA. After the restriction sites have been introduced into the plasmid, the plasmid is cut at these sites to linearize it. A double-stranded oligonucleotide encoding the sequence of the DNA between the restriction sites but containing the desired mutation(s) is synthesized using standard procedures. The two strands are synthesized separately and then hybridized together using standard techniques. This double-stranded oligonucleotide is referred to as the cassette. This cassette is designed to have 3' and 5' ends that are compatible with the ends of the linearized plasmid, such that it can be directly ligated to the plasmid. This plasmid now contains the mutated DNA sequence of GH.

Of course, other methods can be employed to produce GH variants such as the in vitro chemical synthesis of the desired GH variant (Barany et al., *The Peptides*, E. Gross and J. Meienhofer (editors), Academic Press, New York (1979) Volume 2, pp. 3-254).

Production of GH Variants

The GH variants can be conveniently produced by standard recombinant techniques. More specifically, a GH variant can be expressed using a vector-host cell system.

The GH DNA can be inserted into an appropriate plasmid or vector that can subsequently be used to transform a host cell. Prokaryotes are preferred for expressing DNA sequences to produce the GH variants. For example, *E. coli* K12 strain 294 (ATCC No. 31446) can be used, as well as *E. coli* B, *E. coli* X1776 (ATCC No. 31537), *E. coli* c600 and c600hfl, and *E. coli* W3110 (F⁻, γ⁻, prototrophic, ATCC No. 27325), Bacilli such as *Bacillus subtilis*, and other Enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescens*, and various *Pseudomonas* species. A preferred prokaryote is *E. coli* W3110 (ATCC 27325). When expressed intracellularly in prokaryotes, the GH typically contains an N-terminal methionine or a formyl methionine and is not glycosylated. When expressed extracellularly into the medium or the periplasm, the GH does not contain a N-terminal methionine. These examples are, of course, intended to be illustrative rather than limiting.

In addition to prokaryotes, eukaryotic organisms, such as yeast cultures, or cells derived from multicellular organisms, can be used. In principle, any such cell culture is workable. However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture has become a repeatable procedure. Examples of such useful host cell lines are VERO, HeLa, Chinese hamster ovary (CHO), W138, BHK, COS-7, and MDCK cell lines.

In general, plasmid vectors containing replication and control sequences that are derived from species compatible with the host cell are used. The vector ordinarily carries a replication site, as well as sequences that encode proteins that are capable of providing phenotypic selection in transformed cells. For example, *E. coli* can be transformed using pBR322, a plasmid derived from an *E. coli* species (Mandel et al., 1970). Plasmid pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for selection. One preferred vector is pBO475. This vector contains origins of replication for phage and *E. coli* that allow it to be shuttled between such hosts, thereby facilitating mutagenesis and expression. "Expression vector" refers to a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of said DNA in a suitable host. Such

control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, and sequences which control termination of transcription and translation. The vector can be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector can replicate and function independently of the host genome, or can, in some instances, integrate into the genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably as the plasmid is the most commonly used form of vector at present. However, use of other forms of expression vectors which serve equivalent functions and which are, or become, known in the art fall within the scope of the present disclosure.

"Operably linked" when describing the relationship between two DNA or polypeptide regions simply means that they are functionally related to each other. For example, a presequence is operably linked to a peptide if it functions as a signal sequence, participating in the secretion of the mature form of the protein, most probably involving cleavage of the signal sequence. A promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation.

Host cells containing a GH variant expression vector are cultured under conditions suitable for cell growth and for expression of the GH variant. In particular, the culture medium contains appropriate nutrients and growth factors for the host cell employed. The nutrients and growth factors required for growth of a selected host cell are, in many instances, well known or can be readily determined empirically by those skilled in the art. Suitable culture conditions for mammalian host cells, for instance, are described in *Mammalian Cell Culture*, J. P. Mather (editor), Plenum Press (1984) and Barnes and Sato, 1980.

In addition, the culture conditions should allow transcription, translation, and protein transport between cellular compartments. Factors that affect these processes are well-known and include, for example, DNA/RNA copy number; factors that stabilize RNA; nutrients, supplements, and transcriptional inducers or repressors present in the culture medium; temperature, pH, and osmolality of the culture; and cell density. The adjustment of these factors to promote expression in a particular vector-host cell system is within the level of skill in the art.

The cell culture procedure employed in the production of a GH variant can be any of a number of well-known procedures for large- or small-scale production of proteins. These include, but are not limited to, the use of: a fluidized bed bioreactor, a hollow fiber bioreactor, a roller bottle culture system, and a stirred tank bioreactor system. A GH variant can be produced, for instance, in a batch, fed-batch, or continuous mode process.

Methods for recovery of recombinant proteins produced as described above are well-known and vary depending on the expression system employed. For example, if, as is typical, the expression vector contains a signal sequence, the GH variant is recovered from the culture medium or the periplasm. Conveniently, the variant is secreted into the periplasmic space as a fully processed protein (i.e., lacking the secretion signal sequence). However, the GH variant can also be expressed intracellularly and recovered from cell lysates.

The GH variant can be purified from culture medium or a cell lysate by any method capable of separating the variant from components of the host cell or culture medium. Typi-

cally the GH variant is separated from host cell and/or culture medium components that would interfere with pegylation, if desired, or with diagnostic or therapeutic use of the GH variant.

As a first step, the culture medium or cell lysate is usually centrifuged or filtered to remove cellular debris. The supernatant is then typically concentrated or diluted to a desired volume or diafiltered into a suitable buffer to condition the preparation for further purification. Further purification of the GH variant typically includes separating deamidated and clipped forms of the GH variant from the intact form.

In one variation of this embodiment, the GH variant is purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, using a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue stain.

Any of the following exemplary procedures can be employed for purification of a GH variant: affinity chromatography; anion- or cation-exchange chromatography (using, e.g., DEAE SEPHAROSE); chromatography on silica; reverse phase HPLC; gel filtration (using, e.g., SEPHADEX G-75); hydrophobic interaction chromatography; metal-chelate chromatography; ultrafiltration/diafiltration; ethanol precipitation; ammonium sulfate precipitation; chromatofocusing; and displacement chromatography.

Conjugates

GH variants useful in the methods of the present disclosure may be covalently attached (hereinafter "conjugated") to one or more chemical groups. Such conjugation produces a GH variant conjugate having a greater actual molecular weight than the unmodified GH variant. As used herein, the term "actual molecular weight" refers to the molecular weight, as measured by mass spectrometry (e.g., matrix-assisted laser desorption ionization mass spectrometry). The actual molecular weight of the hGH variant conjugate is usually at least about 30 kDa; preferably, in the range of about 35 kDa to about 55 kDa; and more preferably, in the range of about 40 kDa to about 50 kDa. Generally, the actual molecular weight of the hGH variant conjugate does not exceed 100 kDa.

Chemical groups suitable for use in a GH variant conjugate are preferably not significantly toxic or immunogenic, i.e., any toxicity or immunogenicity observed with a GH variant conjugate is not significantly greater (i.e., less than 50%) than any toxicity or immunogenicity observed with the corresponding unmodified GH variant. Typically, a chemical group is selected that reduces toxicity and/or immunogenicity associated with the unmodified GH variant. In addition, the chemical group is conveniently selected to produce a GH variant conjugate that can be stored and used under conditions suitable for storage and use of the unmodified GH variant. Exemplary chemical groups include carbohydrates, such as, for example, those carbohydrates that occur naturally on glycoproteins, and non-proteinaceous polymers, such as polyols.

A polyol, for example, can be conjugated to a GH variant molecule at one or more amino acid residues, including lysine residues, as disclosed in WO 93/00109. The polyol employed can be any water-soluble poly(alkylene oxide) polymer and can have a linear or branched chain. Suitable polyols include those substituted at one or more hydroxyl positions with a chemical group, such as an alkyl group having between one and four carbons. Typically, the polyol is a poly(alkylene glycol), such as poly(ethylene glycol) (PEG), and thus, for ease of description, the remainder of the discussion relates to an exemplary embodiment wherein the

polyol employed is PEG and the process of conjugating the polyol to a GH variant is termed "pegylation." However, those skilled in the art recognize that other polyols, such as, for example, poly(propylene glycol) and polyethylene-polypropylene glycol copolymers, can be employed using the techniques for conjugation described herein for PEG.

The average molecular weight of the PEG can range from about 500 to about 30,000 Da; preferably, from about 1,000 to about 25,000 Da; and more preferably, from about 4,000 to about 20,000 Da. In one embodiment, pegylation is carried out with PEG having an average molecular weight of about 5,000 Da (hereinafter "PEG(5000)"). The reaction conditions are adjusted to maximize production of GH variant molecules conjugated to between about four and about six molecules of PEG(5000). In another embodiment, pegylation is carried out with PEG having an average molecular weight of about 20,000 Da (hereinafter "PEG(20,000)") under conditions adjusted to maximize production of GH molecules conjugated to one molecule of PEG(20,000). In a variation of this embodiment, a branched-chain PEG having two chains of about 10,000 Da each is employed.

PEG preparations that are commercially available, and suitable for use in the present methods, are nonhomogeneous preparations that are sold according to average molecular weight. For example, PEG(5000) preparations typically contain molecules that vary slightly in molecular weight, usually ± 500 Da.

A variety of methods for pegylating proteins have been described (see, for example, U.S. Pat. No. 4,179,337), disclosing the conjugation of a number of hormones and enzymes to PEG and polypropylene glycol to produce physiologically active non-immunogenic compositions. Generally, a PEG having at least one terminal hydroxy group is reacted with a coupling agent to form an activated PEG having a terminal reactive group. This reactive group can then react with the α - and ϵ -amines of proteins to form a covalent bond. Conveniently, the other end of the PEG molecule can be "blocked" with a non-reactive chemical group, such as a methoxy group, to reduce the formation of PEG-crosslinked complexes of protein molecules.

For pegylation of a GH variant, the activated PEG is one that can react with the variant under conditions that do not destroy Site 1 binding activity. Furthermore, activated PEGs that introduce a toxic linking group into the conjugate are usually avoided.

Suitable activated PEGs can be produced by a number of conventional reactions. For example, an N-hydroxysuccinimide ester of a PEG (M-NHS-PEG) can be prepared from PEG-monomethyl ether by reaction with N,N'-dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS), according to the method of Buckmann and Merr, 1981.

In addition, a PEG terminal hydroxy group can be converted to an amino group, for example, by reaction with thionyl bromide to form PEG-Br, followed by aminolysis with excess ammonia to form PEG-NH₂. The PEG-NH₂ is then conjugated to the protein of interest using standard coupling reagents, such as Woodward's Reagent K. Furthermore, a PEG terminal —CH₂OH group can be converted to an aldehyde group, for example, by oxidation with MnO₂. The aldehyde group is conjugated to the protein by reductive alkylation with a reagent such as cyanoborohydride.

Alternatively, activated PEGs suitable for use in the present methods can be purchased.

The degree of pegylation of a GH variant can be adjusted to provide a desirably increased in vivo half-life, compared to the corresponding non-pegylated GH variant. It is believed that the half-life of a pegylated GH variant typi-

cally increases incrementally with increasing degree of pegylation. At higher degrees of pegylation, the increase in half-life of a pegylated GH variant is believed to be partially offset by an increase in the dissociation constant (K_d) for Site 1 binding, indicating a decrease in Site 1 affinity. It is believed that this decrease in affinity is accompanied by a corresponding decrease in potency, which is reflected in an increase in the concentration of conjugate required for 50% maximal effect (EC₅₀). As Site 1 binding is essential for GH antagonist activity of the GH variant, increased pegylation reduces the potency of the GH variants. However, the increase in half-life generally compensates for the reduction in potency, so that the in vivo efficacy of pegylated GH variants is believed to be comparable to, or better than, that observed with the corresponding non-pegylated GH variants. Accordingly, one skilled in the art can readily determine a suitable degree of pegylation for a GH variant to produce a conjugate having a desirably increased half-life, compared to the non-pegylated protein, yet retaining sufficient potency to be efficacious in vivo.

Usually, the half-life is increased at least about five-fold; preferably, at least about 10-fold; more preferably, at least about 50-fold; and most preferably, at least about 100-fold. In addition, the degree and sites of pegylation are such that the PEG-GH variant conjugate is capable of binding GHR at Site 1, typically with a K_d of about 400 nM or lower; preferably, with a K_d of 150 nM or lower; and more preferably, with a K_d of 100 nM or lower, as measured by an equilibrium binding assay, such as that described in Spencer et al., 1988.

The degree and sites of pegylation of a protein are determined by (1) the number and reactivities of pegylation sites (i.e., primary amines) and (2) pegylation reaction conditions. For example, wild-type hGH contains ten primary amines that are theoretically available to react with an activated PEG: the α -amino group of the N-terminal phenylalanine and the ϵ -amino groups of nine lysines. However, because some of the primary amines in hGH and the hGH variants are relatively unreactive, standard pegylation reactions typically result in less than complete pegylation (e.g., seven or eight PEGs per molecule for wild-type hGH).

The sites of pegylation of a protein are also somewhat constrained by the reactivities of the various primary amines. For example, a potential lysine in the Site 1 hormone-receptor binding interface of a given hGH variant may be relatively unreactive with a PEG. Thus, such moderately pegylated hGH variants, having of the order of four to six PEGs per variant molecule, may retain the ability to bind GH receptor at Site 1, despite the presence of a potential pegylation site at this binding interface. In one embodiment, the hGH variant comprises a phenylalanine at position 1 and lysines at positions 38, 120, 140 and 158 conjugated to PEG.

Standard mutagenesis techniques can be used to alter the number of lysines in the protein. Thus, to the extent that amino acid substitutions introduce or replace lysines, GH variants of the present disclosure can contain a greater or lesser number of potential pegylation sites than wild-type GH. In one embodiment, the hGH variant comprises nine potential pegylation sites (Phe1, Lys38, Lys41, Lys70, Lys115, Lys120, Lys140, Lys145, Lys158).

Furthermore, amino acid substitutions introducing or replacing lysines alter the locations of potential pegylation sites. For example, the replacement of G120 with lysine provides an additional potential pegylation site in Site 2, which if pegylated is expected to impair any residual binding at this site.

The degree and sites of pegylation can also be manipulated by adjusting reaction conditions, such as the relative concentrations of the activated PEG and the protein as well as the pH. Suitable conditions for a desired degree of pegylation can be determined empirically.

A composition containing a pegylated GH variant for use in a therapeutic formulation can be heterogeneous or homogeneous, i.e., containing multiple or single pegylated GH variants. Typically, the composition contains at least 70% one or two forms of pegylated GH variants; preferably, at least 80% one or two forms; and more preferably, at least 90% one or two forms.

Antisense Compounds to Growth Hormone Receptor

The methods of the present disclosure rely on the use of an antisense compound to growth hormone receptor (GHR) to modulate growth hormone (GH) signalling or the GH/insulin-like growth factor-I (IGF-I) axis, particularly the expression of GHR and/or IGF-I. Preferably, the antisense compound is an oligonucleotide. However, other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics are contemplated.

Hybridization of an antisense compound with its target nucleic acid is generally referred to as "antisense". Hybridization of the antisense compound with its target nucleic acid inhibits the function of the target nucleic acid. Such "antisense inhibition" is typically based upon hydrogen bonding-based hybridization of the antisense compound to the target nucleic acid such that the target nucleic acid is cleaved, degraded, or otherwise rendered inoperable. The functions of target DNA to be interfered with can include replication and transcription. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of RNA to be interfered with can include functions such as translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA.

"Hybridization" as used herein means pairing of complementary bases of the oligonucleotide and target nucleic acid. Base pairing typically involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases). Guanine (G) and cytosine (C) are examples of complementary nucleobases which pair through the formation of 3 hydrogen bonds. Adenine (A) and thymine (T) are examples of complementary nucleobases which pair through the formation of 2 hydrogen bonds. Hybridization can occur under varying circumstances.

A "nucleoside" is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. "Nucleotides" are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar.

"Specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity such that stable and specific binding occurs between the antisense compound and target nucleic acid. It is understood that the antisense compound need not be 100% complementary to its target nucleic acid sequence to be

specifically hybridizable. An antisense compound is specifically hybridizable when binding of the antisense compound to the target nucleic acid interferes with the expression of the target nucleic acid and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, for example, under physiological conditions in the case of therapeutic treatment.

The term "stringent hybridization conditions" or "stringent conditions" as used herein refers to conditions under which the antisense compound will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent condition under which the antisense compound hybridizes to a target sequence is determined by the nature and composition of the antisense compound and the assays in which it is being investigated.

"Complementary" as used herein, refers to the capacity for precise pairing between a nucleobase of the antisense compound and the target nucleic acid. For example, if a nucleobase at a certain position of the antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of the target nucleic acid, then the position of hydrogen bonding between the antisense compound and the target nucleic acid is considered to be a complementary position. The antisense compound may hybridize over one or more segments, such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure). In one embodiment, the antisense compound comprises at least 70% sequence complementarity to a target region within the target nucleic acid. For example, an antisense compound in which 18 of 20 nucleobases are complementary to a target region within the target nucleic acid, and would therefore specifically hybridize, would represent 90% complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other, or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 non-complementary nucleobases which are flanked by 2 regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus, fall within the scope of the present disclosure. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., 1990; Zhang and Madden, 1997).

Antisense Oligonucleotides

The present disclosure provides for use of an antisense oligonucleotide for inhibiting expression of a growth hormone receptor (GHR).

The term "inhibits" as used herein means any measurable decrease (e.g., 10%, 20%, 50%, 90%, or 100%) in GHR expression.

As used herein, the term "oligonucleotide" refers to an oligomer or polymer of RNA or DNA or mimetics, chimeras, analogs and homologs thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages, as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for

example, enhanced cellular uptake, enhanced affinity for the target nucleic acid and increased stability in the presence of nucleases.

In forming oligonucleotides, phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound; however, linear compounds are generally preferred. In addition, linear compounds may have internal nucleobase complementarity and may therefore fold in a manner so as to produce a fully or partially double-stranded compound. With regard to oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Antisense oligonucleotides useful in the methods of the present disclosure include, for example, ribozymes, siRNA, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligonucleotides which hybridize to at least a portion of the target nucleic acid.

Antisense oligonucleotides may be administered in the form of single-stranded, double-stranded, circular or hairpin and may contain structural elements such as internal or terminal bulges or loops. Once administered, the antisense oligonucleotides may elicit the action of one or more enzymes or structural proteins to effect modification of the target nucleic acid.

One non-limiting example of such an enzyme is RNase H, a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H. Activation of RNase H therefore results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. Similar roles have been postulated for other ribonucleases, such as those in the RNase III and ribonuclease L family of enzymes.

The introduction of double-stranded RNA (dsRNA) molecules, has been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products. This phenomenon occurs in both plants and animals and is believed to have an evolutionary connection to viral defense and transposon silencing.

The first evidence that dsRNA could lead to gene silencing in animals came in 1995 from work in the nematode, *Caenorhabditis elegans* (Guo and Kempheus, 1995). Montgomery et al. (1998) have shown that the primary interference effects of dsRNA are posttranscriptional. The posttranscriptional antisense mechanism defined in *Caenorhabditis elegans* resulting from exposure to double-stranded RNA (dsRNA) has since been designated RNA interference (RNAi). This term has been generalized to mean antisense-mediated gene silencing involving the introduction of dsRNA leading to the sequence-specific reduction of endogenous targeted mRNA levels (Fire et al., 1998). It has been shown that it is, in fact, the single-stranded RNA oligomers of antisense polarity of the dsRNAs which are the potent inducers of RNAi (Tijsterman et al., 2002).

A person having ordinary skill in the art could, without undue experimentation, identify antisense oligonucleotides useful in the methods of the present disclosure.

Modified Internucleoside Linkages (Backbones)

Antisense compounds useful in the methods of the present disclosure include oligonucleotides having modified backbones or non-natural internucleoside linkages. Oligonucleotides having modified backbones include those that retain

a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone.

Modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage.

Oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage, that is, a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808, 4,469,863, 4,476,301, 5,023,243, 5,177,196, 5,188,897, 5,264,423, 5,276,019, 5,278,302, 5,286,717, 5,321,131, 5,399,676, 5,405,939, 5,453,496, 5,455,233, 5,466,677, 5,476,925, 5,519,126, 5,536,821, 5,541,306, 5,550,111, 5,563,253, 5,571,799, 5,587,361, 5,194,599, 5,565,555, 5,527,899, 5,721,218, 5,672,697 and 5,625,050.

Modified oligonucleotide backbones that do not include a phosphorus atom therein include, for example, backbones formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

Representative United States patents that teach the preparation of the above oligonucleotides include, but are not limited to, U.S. Pat. Nos. 5,034,506, 5,166,315, 5,185,444, 5,214,134, 5,216,141, 5,235,033, 5,264,562, 5,264,564, 5,405,938, 5,434,257, 5,466,677, 5,470,967, 5,489,677, 5,541,307, 5,561,225, 5,596,086, 5,602,240, 5,610,289, 5,602,240, 5,608,046, 5,610,289, 5,618,704, 5,623,070, 5,663,312, 5,633,360, 5,677,437, 5,792,608, 5,646,269 and 5,677,439.

Modified Sugar and Internucleoside Linkages

Antisense compounds useful in the methods of the present disclosure include oligonucleotide mimetics where both the sugar and the internucleoside linkage (i.e. the backbone) of the nucleotide units are replaced with novel groups. The nucleobase units are maintained for hybridization with the target nucleic acid.

An oligonucleotide mimetic that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular, an aminoethylglycine back-

bone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082, 5,714,331, and 5,719,262. Further teaching of PNA compounds can be found in Nielsen et al., 1991.

The antisense compounds useful in the methods of the present disclosure also include oligonucleotides with phosphorothioate backbones and oligonucleotides with heteroatom backbones, for example, $-\text{CH}_2-\text{NH}-\text{O}-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{O}-\text{CH}_2-$ [known as a methylene (methylimino) or MMI backbone], $-\text{CH}_2-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{N}(\text{CH}_3)-\text{CH}_2-$ and $-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-$ [wherein the native phosphodiester backbone is represented as $-\text{O}-\text{P}-\text{O}-\text{CH}_2-$] of U.S. Pat. No. 5,489,677, and the amide backbones of U.S. Pat. No. 5,602,240.

The antisense compounds useful in the methods of the present disclosure also include oligonucleotides having morpholino backbone structures of U.S. Pat. No. 5,034,506. Modified Sugars

Antisense compounds useful in the methods of the present disclosure include oligonucleotides having one or more substituted sugar moieties.

Examples include oligonucleotides comprising one of the following at the 2' position: OH; F; O—, S—, or N-alkyl; O—, S—, or N-alkenyl; O—, S— or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl.

In one embodiment, the oligonucleotide comprises one of the following at the 2' position: $\text{O}[(\text{CH}_2)_n\text{O}]_m\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{OCH}_3$, $\text{O}(\text{CH}_2)_n\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{ONH}_2$, and $\text{O}(\text{CH}_2)_n\text{ON}[(\text{CH}_2)_m\text{CH}_3]_2$, where n and m are from 1 to about 10.

Further examples include of modified oligonucleotides include oligonucleotides comprising one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3 , OCN , Cl, Br, CN, CF_3 , OCF_3 , SOCH_3 , SO_2CH_3 , ONO_2 , NO_2 , N_3 , NH_2 , heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties.

In one embodiment, the modification includes 2'-methoxyethoxy (2'-O— $\text{CH}_2\text{CH}_2\text{OCH}_3$ (also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., 1995), that is, an alkoxyalkoxy group. In a further embodiment, the modification includes 2'-dimethylaminoethoxyethoxy, that is, a $\text{O}(\text{CH}_2)_2\text{ON}(\text{CH}_3)_2$ group (also known as 2'-DMAOE), or 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), that is, 2'-O— $\text{CH}_2-\text{O}-\text{CH}_2-\text{N}(\text{CH}_3)_2$.

Other modifications include 2'-methoxy (2'-O— CH_3), 2'-aminopropoxy (2'- $\text{OCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$), 2'-allyl (2'- $\text{CH}_2-\text{CH}=\text{CH}_2$), 2'-O-allyl (2'-O— $\text{CH}_2-\text{CH}=\text{CH}_2$) and 2'-fluoro (2'-F). The 2'-modification may be in the arabino

(up) position or ribo (down) position. In one embodiment a 2'-arabino modification is 2'-F.

Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of the 5' terminal nucleotide.

Oligonucleotides may also have sugar mimetics, such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957, 5,118,800, 5,319,080, 5,359,044, 5,393,878, 5,446,137, 5,466,786, 5,514,785, 5,519,134, 5,567,811, 5,576,427, 5,591,722, 5,597,909, 5,610,300, 5,627,053, 5,639,873, 5,646,265, 5,658,873, 5,670,633, 5,792,747, and 5,700,920.

A further modification of the sugar includes Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 3' or 4' carbon atom of the sugar ring, thereby forming a bicyclic sugar moiety. In one embodiment, the linkage is a methylene ($-\text{CH}_2-$)_n group bridging the 2' oxygen atom and the 4' carbon atom, wherein n is 1 or 2. LNAs and preparation thereof are described in WO 98/39352 and WO 99/14226.

25 Natural and Modified Nucleobases

Antisense compounds useful in the methods of the present disclosure include oligonucleotides having nucleobase modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

Modified nucleobases include other synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl ($-\text{C}\equiv\text{C}-\text{CH}_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Further modified nucleobases include tricyclic pyrimidines, such as phenoxazine cytidine (1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as, for example, a substituted phenoxazine cytidine (e.g., 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one).

Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example, 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in J. I. Kroschwitz (editor), The Concise Encyclopedia of Polymer Science and Engineering, pages 858-859, John Wiley and Sons (1990), those disclosed by Englisch et

al. (1991), and those disclosed by Y. S. Sanghvi, Chapter 15: Antisense Research and Applications, pages 289-302, S. T. Crooke, B. Lebleu (editors), CRC Press, 1993.

Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligonucleotide. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-amino-propyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. In one embodiment, these nucleobase substitutions are combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, U.S. Pat. Nos. 3,687,808, 4,845,205, 5,130,302, 5,134,066, 5,175,273, 5,367,066, 5,432,272, 5,457,187, 5,459,255, 5,484,908, 5,502,177, 5,525,711, 5,552,540, 5,587,469, 5,594,121, 5,596,091, 5,614,617, 5,645,985, 5,830,653, 5,763,588, 6,005,096, 5,681,941 and 5,750,692. Conjugates

Antisense compounds useful in the methods of the present disclosure may be conjugated to one or more moieties or groups which enhance the activity, cellular distribution or cellular uptake of the antisense compound.

These moieties or groups may be covalently bound to functional groups such as primary or secondary hydroxyl groups.

Exemplary moieties or groups include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugate groups include cholesterol, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins and dyes.

Moieties or groups that enhance the pharmacodynamic properties include those that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid.

Moieties or groups that enhance the pharmacokinetic properties include those that improve uptake, distribution, metabolism or excretion of the antisense compounds.

Representative moieties or groups are disclosed in PCT/US92/09196 and U.S. Pat. No. 6,287,860.

Moieties or groups include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, for example, hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, for example, dodecandiol or undecyl residues, a phospholipid, for example, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycerol-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmitoyl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

Antisense compounds useful in the methods of the present disclosure may also be conjugated to active drug substances.

Oligonucleotide-drug conjugates and their preparation are described in U.S. Ser. No. 09/334,130.

Representative United States patents that teach the preparation of such conjugates include, but are not limited to, U.S.

Pat. Nos. 4,828,979, 4,948,882, 5,218,105, 5,525,465, 5,541,313, 5,545,730, 5,552,538, 5,578,717, 5,580,731, 5,580,731, 5,591,584, 5,109,124, 5,118,802, 5,138,045, 5,414,077, 5,486,603, 5,512,439, 5,578,718, 5,608,046, 4,587,044, 4,605,735, 4,667,025, 4,762,779, 4,789,737, 4,824,941, 4,835,263, 4,876,335, 4,904,582, 4,958,013, 5,082,830, 5,112,963, 5,214,136, 5,082,830, 5,112,963, 5,214,136, 5,245,022, 5,254,469, 5,258,506, 5,262,536, 5,272,250, 5,292,873, 5,317,098, 5,371,241, 5,391,723, 5,416,203, 5,451,463, 5,510,475, 5,512,667, 5,514,785, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,595,726, 5,597,696, 5,599,923, 5,599,928 and 5,688,941. Chimeric Compounds

As would be appreciated by those skilled in the art, it is not necessary for all positions in a given compound to be uniformly modified and in fact, more than one of the aforementioned modifications may be incorporated in a single oligonucleotide or even at a single nucleoside within an oligonucleotide.

Antisense compounds useful in the methods of the present disclosure include chimeric oligonucleotides. "Chimeric oligonucleotides" contain two or more chemically distinct regions, each made up of at least one monomer unit, that is, a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, increased stability and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. The cleavage of RNA:RNA hybrids can, in like fashion, be accomplished through the actions of endoribonucleases, such as RNaseL which cleaves both cellular and viral RNA. Cleavage of the RNA target can be routinely detected by gel electrophoresis and if necessary, associated nucleic acid hybridization techniques known in the art.

Chimeric antisense compounds useful in the methods of the present disclosure may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, and/or oligonucleotide mimetics. Such compounds have also been referred to in the art as hybrids or gapmers.

Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830, 5,149,797, 5,220,007, 5,256,775, 5,366,878, 5,403,711, 5,491,133, 5,565,350, 5,623,065, 5,652,355, 5,652,356, and 5,700,922.

Exemplary Oligonucleotides

In one embodiment, the antisense compound is a second generation phosphorothioate backbone 2'-MOE-modified chimeric oligonucleotide gapmer designed to hybridize to GHR mRNA.

Exemplary oligonucleotides are shown in Table 1. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the oligonucleotide binds. "% Inhib" indicates the inhibitory effect on hGHR mRNA levels by quantitative real-time PCR. Data are averages from three experiments in which MCF7 cells were treated with the antisense oligonucleotides.

TABLE 1

Inhibition of human growth hormone receptor mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap						
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
227452	Coding	4	332	tcagggcattctttccattc	79	6
227453	Coding	4	337	cataatcagggcattctttc	52	7
227464	Coding	4	947	cctttaatctttggaactgg	58	8
227468	Coding	4	1079	tcatcaatatctagctcaat	62	9
227469	Coding	4	1124	cttagaagctgtctgtgtc	63	10
227475	Coding	4	1514	cctgctgggtgaatgtcgct	68	11
227480	Coding	4	1724	atgtaaatgtcctcttggtt	66	12
227481	Coding	4	1729	tggtgatgtaaatgtcctct	45	13
227482	Coding	4	1734	ttctgtgggtgatgtaaatgt	53	14
227483	Coding	4	1739	aggctttctgtggtgatgta	75	15
227484	Coding	4	1744	tggtgaaggctttctgtggtg	63	16
227488	Coding	4	1922	agttggtctgtgctcacata	86	17
227489	Coding	4	1927	tttcagttggtctgtgctc	75	18
227490	Coding	4	1936	gcatgattttttcagttgg	67	19
227499	3'UTR	4	2656	tataaaagggtttgtaaaa	14	20
227500	3'UTR	4	4043	catagcagcaaagtagcaga	69	21
227501	3'UTR	4	4183	gctatttttggctatagaaa	64	22
227502	3'UTR	4	4197	gattgagggtatttagctatt	56	23
272302	Start Codon	4	31	gatccatacctgtaggacct	60	24
272303	Start Codon	4	36	ccagagatccatacctgtag	55	25
272304	Coding	4	115	tgctaaggatagctgctgtg	48	26
272305	Coding	4	160	ttgtctttaggcctggatta	68	27
272306	Coding	4	170	ttagaagaatttgtcttttag	13	28
272307	Coding	4	185	gtgaatttaggctccttaga	55	29
272308	Coding	4	274	gctgtatgggtcctagggttc	57	30
272309	Coding	4	362	taacagctgttttccccagc	85	31
272310	Coding	4	439	tttcattccactgtaccacca	76	32
272311	Coding	4	468	ttgcactattttcatcaacag	47	33
272312	Coding	4	480	gggtggatctggttgacta	57	34
272313	Coding	4	564	attgctggtgcttcccatc	77	35
272314	Coding	4	652	taggggtccatcattttccat	56	36
272315	Coding	4	684	caatgagtacactggaactg	53	37
272316	Coding	4	752	aactcgccataatttccaga	64	38
272317	Coding	4	857	agcccaaatattccaaagat	65	39
272318	Coding	4	913	tcagcatttttaatcctttgc	55	40

TABLE 1-continued

Inhibition of human growth hormone receptor mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap						
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
272319	Coding	4	979	atcttccttccttgaggaga	67	41
272320	Coding	4	1000	agattgtgttcacctcctct	70	42
272321	Coding	4	1053	aacccaagagtcactcactgt	64	43
272322	Coding	4	1084	ctggctcatcaatatctagc	84	44
272323	Coding	4	1110	tgtgtctgattcctcagtct	67	45
272324	Coding	4	1236	tatgtcattggcattgaaat	53	46
272325	Coding	4	1302	aaggcataagagatctgctt	66	47
272326	Coding	4	1420	actcagctccttcagtagga	77	48
272327	Coding	4	1560	ggacatccctgccttattct	60	49
272328	Coding	4	1623	ggcattgtccataaggaagt	85	50
272329	Coding	4	1651	actttttggcatctgcctca	63	51
272330	Coding	4	1656	gatgcactttttggcatctg	47	52
272331	Coding	4	1861	cagtcgcattgagtatgagg	67	53
272332	Coding	4	1884	ctctttgtcaggcaagggca	75	54
272333	Coding	4	1913	gtgctcacatagccacatga	72	55
272334	Stop Codon	4	1949	aagaaaggctaaggcatgat	61	56
272335	3'UTR	4	1973	aaatacgtagctcttgggaa	47	57
272336	3'UTR	4	2196	caatcactgctactaaacag	69	58
272337	3'UTR	4	2249	aaacatagccattcaatgct	39	59
272338	3'UTR	4	2337	gtgctatggtttgcattcaa	78	60
272339	3'UTR	4	2454	gttttacatatccaaactat	72	61
272340	3'UTR	4	2853	catcaaccaagatttggtga	69	62
272341	3'UTR	4	2988	gaggctatagatcttatctc	65	63
272342	3'UTR	4	3271	tagtgagaagaaagtttct	45	64
272343	3'UTR	4	3765	aatgctctcaagaatgatgt	48	65
272344	3'UTR	4	3980	acactcaattctagcttttc	60	66
272345	3'UTR	4	4011	catctattacaataacatg	24	67
272346	3'UTR	4	4057	ctcttgagaaaaccatagc	67	68
272347	3'UTR	4	4097	tctacactgatgatacttta	62	69
272348	3'UTR	4	4120	cacagctttgaattgaatta	57	70
272349	3'UTR	4	4133	agtcttccaaacacacagct	68	71
272350	3'UTR	4	4156	aggctgtgtgaaatagtaa	67	72
272351	3'UTR	4	4170	atagaaatgtgtcaggctg	57	73
272352	3'UTR	4	4218	ccaaaatgacattctgagac	77	74
272353	3'UTR	4	4245	ataatggcttatgtggccac	72	75
272354	intron	5	2571	agttatgtgacctgattga	65	76

TABLE 1-continued

Inhibition of human growth hormone receptor mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap						
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
272355	intron: exon junction	5	6418	ttgagtggttcctaaaatgaa	24	77
272356	intron	5	8405	atggaggctggaggttcaaa	63	78
272357	intron: exon junction	5	22712	taggggtccatctttcaagac	62	79
272358	intron	5	25543	tctccagatagaatctaaac	53	80
272359	intron	5	29755	tccaaatattctggtacttt	72	81
272360	exon: intron junction	5	29935	tattagttaccttgaggaga	0	82
272361	intron: exon junction	5	30267	attttccttcctagaaaata	10	83

All oligonucleotides in Table 1 are chimeric oligonucleotides ("gapmers"), 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All uracils are 5-methyluracils (^{Me}U). Typically, the oligonucleotide is synthesized using 2-methoxyethyl modified thymidines not 5-methyluracils. All pyrimidines are C5 methylated (i.e., U, T, C are C5 methylated).

The oligonucleotide may be synthesized by a multi-step process that may be divided into two distinct operations: solid-phase synthesis and downstream processing. In the first operation, the nucleotide sequence of the oligonucleotide is assembled through a computer-controlled solid-phase synthesizer. Subsequent downstream processing includes deprotection steps, preparative reversed-phase chromatographic purification, isolation and drying to yield the oligonucleotide drug substance. The chemical synthesis of the oligonucleotide utilizes phosphoramidite coupling chemistry followed by oxidative sulfurization and involves sequential coupling of activated monomers to an elongating oligomer, the 3'-terminus of which is covalently attached to the solid support.

Detritylation (reaction a).

Each cycle of the solid-phase synthesis commences with removal of the acid-labile 5'-O-4, 4'-dimethoxytrityl (DMT) protecting group of the 5' terminal nucleoside of the support bound oligonucleotide. This is accomplished by treatment with an acid solution (e.g., dichloroacetic acid (DCA) in toluene). Following detritylation, excess reagent is removed from the support by washing with acetonitrile in preparation for the next reaction.

Coupling (Reaction b)

Chain elongation is achieved by reaction of the 5'-hydroxyl group of the support-bound oligonucleotide with a

solution of the phosphoramidite corresponding to that particular base position (e.g., for base2: MOE-^{Me}C amidite) in the presence of an activator (e.g., 1H-tetrazole). This results in the formation of a phosphite triester linkage between the incoming nucleotide synthon and the support-bound oligonucleotide chain. After the coupling reaction, excess reagent is removed from the support by washing with acetonitrile in preparation for the next reaction.

Sulfurization (Reaction c)

The newly formed phosphite triester linkage is converted to the corresponding [O, O, O]-trialkyl phosphorothioate triester by treatment with a solution of a sulfur transfer reagent (e.g., phenylacetyl disulfide). Following sulfurization, excess reagent is removed from the support by washing with acetonitrile in preparation for the next reaction.

Capping (Reaction d)

A small proportion of the 5'-hydroxy groups available in any given cycle fail to extend. Coupling of these groups in any of the subsequent cycles would result in formation of process-related impurities ("DMT-on (n-1)-mers") which are difficult to separate from the desired product. To prevent formation of these impurities and to facilitate purification, a "capping reagent" (e.g., acetic anhydride and N-methylimidazole/acetonitrile/pyridine) is introduced into the reactor vessel to give capped sequences. The resulting failure sequences ("DMT-off shortmers") are separated from the desired product by reversed phase HPLC purification. After the capping reaction, excess reagent is removed from the support by washing with acetonitrile in preparation of the next reaction.

Reiteration of this basic four-step cycle using the appropriate protected nucleoside phosphoramidite allows assembly of the entire protected oligonucleotide sequence.

Backbone Deprotection (Reaction e)

Following completion of the assembly portion of the process the cyanoethyl groups protecting the (O, O, O)-trialkyl phosphorothioate triester internucleotide linkages are removed by treatment with a solution of triethylamine

(TEA) in acetonitrile. The reagent and acrylonitrile generated during this step are removed by washing the column with acetonitrile.

Cleavage from Support and Base Deprotection (Reaction f)

Deprotection of the exocyclic amino groups and cleavage of the crude product from the support is achieved by incubation with aqueous ammonium hydroxide (reaction f). Purification of the crude, 5'-O-DMT-protected product is accomplished by reversed phase HPLC. The reversed phase HPLC step removes DMT-off failure sequences. The elution profile is monitored by UV absorption spectroscopy. Fractions containing DMT-on oligonucleotide product are collected and analyzed.

Acidic Deprotection (Reaction g)

Reversed phase HPLC fractions containing 5'-O-DMT-protected oligonucleotide are pooled and transferred to a precipitation tank. The products obtained from the purification of several syntheses are combined at this stage of the process. Purified DMT-on oligonucleotide is treated with acid (e.g., acetic acid) to remove the DMT group attached to the 5' terminus. After acid exposure for the prescribed time and neutralization, the oligonucleotide drug substance is isolated and dried.

Following the final acidic deprotection step, the solution is neutralized by addition of aqueous sodium hydroxide and the oligonucleotide drug substance is precipitated from solution by adding ethanol. The precipitated material is allowed to settle at the bottom of the reaction vessel and the ethanolic supernatant decanted. The precipitated material is redissolved in purified water and the solution pH adjusted to between pH 7.2 and 7.3. The precipitation step is repeated. The precipitated material is dissolved in water and the solution filtered through a 0.45 micron filter and transferred into disposable polypropylene trays that are then loaded into a lyophilizer. The solution is cooled to -50°C . Primary drying is carried out at 25°C . for 37 hours. The temperature is increased to 300°C . and a secondary drying step performed for 5.5 hours. Following completion of the lyophilization process, the drug substance is transferred to high density polyethylene bottles and stored at -200°C .
Target Nucleic Acid

"Targeting" an antisense compound to a particular nucleic acid can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. In the present disclosure, the target nucleic acid encodes growth hormone receptor (GHR). The term "target nucleic acid" encompasses DNA encoding GHR, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and further, cDNA derived from such RNA.

The cDNA encoding the growth hormone receptor has been cloned from many species. The receptor consists of an extracellular hormone-binding region (exons 2-7), a single membrane spanning region (exon 8), and an intracellular region (exons 9-10). There are also multiple alternative 5' untranslated regions which are alternative first exons of the gene, in both the human and mouse transcripts. Growth hormone receptor has no intrinsic kinase domain, but the intracellular region plays a major role in the signal transduction process. A truncated form of the receptor, known as growth hormone binding protein (GHBP), lacks the transmembrane and intracellular regions of GHR and is secreted into the serum. The truncated protein is produced by one of two different processes, depending on the animal species. In mice and rats, alternative splicing of GHR precursor messenger RNA replaces the transmembrane and intracellular

regions with a very short hydrophilic tail (encoded by exon 8A). In humans, cows, and pigs (among others), no alternative RNA splicing is apparent but instead the GHBP is produced by proteolysis of the GHR. The GHBP appears to be to modulate the level of circulating growth hormone (GH).

In one embodiment the GHR is a human GHR (hGHR) having a nucleotide sequence as shown in NM_000163.4 (SEQ ID NO:4) or NG_011688 (4852-302955) (SEQ ID NO:5).

The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, for example, inhibition of expression, will result. The term "region" as used herein is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of the target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites" as used herein, means positions within the target nucleic acid.

Since the "translation initiation codon" is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon", the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG, or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. The terms "start codon" and "translation initiation codon" as used herein refer to the codon or codons that are used in vivo to initiate translation of an mRNA transcribed from a gene encoding, for example, GHR, regardless of the sequence(s) of such codons.

A "translation termination codon" also referred to as a "stop codon" may have one of three RNA sequences: 5'-UAA, 5'-UAG and 5'-UGA (5'-TAA, 5'-TAG and 5'-TGA, respectively in the corresponding DNA molecule). The terms "translation termination codon" and "stop codon" as used herein refer to the codon or codons that are used in vivo to terminate translation of an mRNA transcribed from a gene encoding the GHR, regardless of the sequence(s) of such codons.

The terms "start codon region" and "translation initiation codon region" refer to a portion of the mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from the translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of the mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from the translation termination codon. Consequently, the "start codon region" or "translation initiation codon region" and the "stop codon region" or "translation termination codon region" are all regions which may be targeted effectively with the antisense compounds.

The "open reading frame" (ORF) or "coding region", which is known in the art to refer to the region between the translation initiation codon and the translation termination

codon, is also a region which may be targeted effectively. In one embodiment, the intragenic region encompassing the translation initiation or termination codon of the ORF of a gene is targeted.

Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of the mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of the mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3'UTR), known in the art to refer to the portion of the mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of the mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself, as well as the first 50 nucleotides adjacent to the cap site. In one embodiment, the 5' cap region is targeted.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts". In one embodiment, introns, or splice sites, that is, intron-exon junctions or exon-intron junctions, or aberrant fusion junctions due to rearrangements or deletions are targeted.

Alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants".

"Pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence. Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

In mouse, rat and monkey, GHBP, which is the soluble shortened form of GHR, is produced by alternative splicing of the GHR primary transcript. In some embodiments it may be preferable to target regions of the transcript which are present in both the GHR transcript and in the shorter GHBP transcript. In other embodiments it may be preferable to target regions of the mRNA which are only present in the longer GHR transcript. In humans, cows, and pigs (among others), no alternative RNA splicing is apparent but instead the shorter GHBP is produced by proteolysis of the GHR. It will be understood that in the context of this disclosure, "nucleic acid encoding GHR" includes nucleic acid encoding GHBP.

Variants can be produced through the use of alternative signals to start or stop transcription, that is, through use of an alternative start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. In one embodiment, the pre-mRNA or mRNA variants are targeted. The human GHR has several transcript variants as can be identified from the National Center for Biotechnology Information <http://www.ncbi.nlm.nih.gov/guide/and> other web sites http://www.uniprot.org/uniprot/P10912#PRO_0000010958. There are additionally alternative sequences and natural variants sequences of these transcripts.

The location on the target nucleic acid to which the antisense compound hybridizes is referred to as the "target segment". As used herein, the term "target segment" is defined as at least an 8-nucleobase portion of a target region to which an antisense compound is targeted. While not wishing to be bound by theory, it is presently believed that these target segments represent portions of the target nucleic acid which are accessible for hybridization.

Once one or more target regions, segments or sites have been identified, antisense compounds are chosen which are sufficiently complementary to a target segment, that is, antisense compounds that hybridize sufficiently well and with sufficient specificity, to give the desired effect.

In a further embodiment, the target segment identified herein may be employed in a screen for additional compounds that modulate the expression of the GHR gene (and thus expression of GHR). "Modulators" are those compounds that decrease or increase the expression of a nucleic acid molecule encoding GHR and which comprise at least a 8 nucleobase portion which is complementary to a preferred target segment.

The screening method comprises the steps of contacting a target segment of the nucleic acid encoding GHR with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid encoding GHR. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g., either decreasing or increasing) the expression of a nucleic acid encoding GHR, the modulator may then be employed in further investigative studies of the function of GHR, or for use as a research, diagnostic, or therapeutic agent.

The target segment may also be combined with its respective complementary antisense compound to form stabilized double-stranded (duplexed) oligonucleotides.

Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation, as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications (Fire et al., 1998; Timmons and Fire, 1998; Timmons et al., 2001; Tabara et al., 1998; Montgomery et al., 1998; Tuschl et al., 1999; Elbashir et al., 2001a; Elbashir et al., 2001b). For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target (Tijsterman et al., 2002).

Exemplary Target Nucleic Acids

Exemplary target sequences are shown in Table 2.

TABLE 2

Sequence and position of preferred target segments identified in growth hormone receptor						
SITE ID	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	REV COMP OF SEQ ID	ACTIVE IN	SEQ ID NO
144070	4	332	gaatggaaagaatgccctga	6	<i>H. sapiens</i>	84
144071	4	337	gaaagaatgccctgattatg	7	<i>H. sapiens</i>	85
144082	4	947	ccagttccaagattaaagg	8	<i>H. sapiens</i>	86
144086	4	1079	attgagctagatattgatga	9	<i>H. sapiens</i>	87
144087	4	1124	gacacagacagacttctaag	10	<i>H. sapiens</i>	88
144093	4	1514	agcgacattacaccagcagg	11	<i>H. sapiens</i>	89
144098	4	1724	aaccaagaggacatttacat	12	<i>H. sapiens</i>	90
144099	4	1729	agaggacatttacatcacca	13	<i>H. sapiens</i>	91
144100	4	1734	acatttacatcaccacagaa	14	<i>H. sapiens</i>	92
144101	4	1739	tacatcaccacagaaagcct	15	<i>H. sapiens</i>	93
144102	4	1744	caccacagaaagccttacca	16	<i>H. sapiens</i>	94
144106	4	1922	tatgtgagcacagaccaact	17	<i>H. sapiens</i>	95
144107	4	1927	gagcacagaccaactgaaca	18	<i>H. sapiens</i>	96
144108	4	1936	ccaactgaacaaaatcatgc	19	<i>H. sapiens</i>	97
144118	4	4043	tctgctactttgctgctatg	21	<i>H. sapiens</i>	98
144119	4	4183	tttctatagccaaaaatagc	22	<i>H. sapiens</i>	99
144120	4	4197	aatagctaaatacctcaatc	23	<i>H. sapiens</i>	100
188518	4	31	aggtcctacaggatggatc	24	<i>H. sapiens</i>	101
188519	4	36	ctacaggatggatctctgg	25	<i>H. sapiens</i>	102
188520	4	115	cacagcagctatccttagca	26	<i>H. sapiens</i>	103
188521	4	160	taatccaggcctaagacaa	27	<i>H. sapiens</i>	104
188523	4	185	tctaaggagcctaattcac	29	<i>H. sapiens</i>	105
188524	4	274	gaacctaggaccatacagc	30	<i>H. sapiens</i>	106
188525	4	362	gctggggaaaacagctgtta	31	<i>H. sapiens</i>	107
188526	4	439	tggtggtacagtggatgaaa	32	<i>H. sapiens</i>	108
188527	4	468	ctgttgatgaaatagtgcaa	33	<i>H. sapiens</i>	109
188528	4	480	tagtgcaaccagatccaccc	34	<i>H. sapiens</i>	110
188529	4	564	gatgggaagcaccacgcaat	35	<i>H. sapiens</i>	111
188530	4	652	atggaaaatgatggacccta	36	<i>H. sapiens</i>	112
188531	4	684	cagttccagtgtactcattg	37	<i>H. sapiens</i>	113
188532	4	752	tctggaaattatggcgagtt	38	<i>H. sapiens</i>	114
188533	4	857	atctttggaatatattgggct	39	<i>H. sapiens</i>	115
188534	4	913	gcaaaggattaaaatgctga	40	<i>H. sapiens</i>	116
188535	4	979	tctcctcaaggaaggaaaat	41	<i>H. sapiens</i>	117
188536	4	1000	agaggaggtgaacacaatct	42	<i>H. sapiens</i>	118

TABLE 2-continued

Sequence and position of preferred target segments identified in growth hormone receptor						
SITE ID	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	REV COMP OF SEQ ID	ACTIVE IN	SEQ ID NO
188537	4	1053	acagtgatgactcttgggtt	43	<i>H. sapiens</i>	119
188538	4	1084	gctagatattgatgagccag	44	<i>H. sapiens</i>	120
188539	4	1110	agactgaggaatcagacaca	45	<i>H. sapiens</i>	121
188540	4	1236	atttcaatgccaatgacata	46	<i>H. sapiens</i>	122
188541	4	1302	aagcagatctcttatgcctt	47	<i>H. sapiens</i>	123
188542	4	1420	tcctactgaaggagctgagt	48	<i>H. sapiens</i>	124
188543	4	1560	agaataaggcagggatgtcc	49	<i>H. sapiens</i>	125
188544	4	1623	acttccttatggacaatgcc	50	<i>H. sapiens</i>	126
188545	4	1651	tgaggcagatgcaaaaagt	51	<i>H. sapiens</i>	127
188546	4	1656	cagatgcaaaaagtgcatc	52	<i>H. sapiens</i>	128
188547	4	1861	cctcatactcaatgcgactg	53	<i>H. sapiens</i>	129
188548	4	1884	tgcccttgctgacaaagag	54	<i>H. sapiens</i>	130
188549	4	1913	tcatgtggctatgtgagcac	55	<i>H. sapiens</i>	131
188550	4	1949	atcatgccttagcctttctt	56	<i>H. sapiens</i>	132
188551	4	1973	ttcccaagagctacgtat	57	<i>H. sapiens</i>	133
188552	4	2196	ctgttttagtagcagtgattg	58	<i>H. sapiens</i>	134
188554	4	2337	ttgaatgcaaaccatagcac	60	<i>H. sapiens</i>	135
188555	4	2454	atagtttgatgatgtaaac	61	<i>H. sapiens</i>	136
188556	4	2853	tcaccaaactcttggtgatg	62	<i>H. sapiens</i>	137
188557	4	2988	gagataagatctatagcctc	63	<i>H. sapiens</i>	138
188558	4	3271	agaaactttctttctcacta	64	<i>H. sapiens</i>	139
188559	4	3765	acatcattcttgagagcatt	65	<i>H. sapiens</i>	140
188560	4	3980	gaaaagctagaattgagtgt	66	<i>H. sapiens</i>	141
188562	4	4057	gctatggttttctccaagag	68	<i>H. sapiens</i>	142
188563	4	4097	taaagtatcatcagtgtaga	69	<i>H. sapiens</i>	143
188564	4	4120	taattcaattcaaagctgtg	70	<i>H. sapiens</i>	144
188565	4	4133	agctgtgtgtttggaagact	71	<i>H. sapiens</i>	145
188566	4	4156	ttactatttcacaacagcct	72	<i>H. sapiens</i>	146
188567	4	4170	cagcctgacaacatttctat	73	<i>H. sapiens</i>	147
188568	4	4218	gtctcagaatgtcattttgg	74	<i>H. sapiens</i>	148
188569	4	4245	gtggccacataagccattat	75	<i>H. sapiens</i>	149
188570	5	2571	tcaatcagggtcacataact	76	<i>H. sapiens</i>	150
188572	5	8405	tttgaacctccagcctccat	78	<i>H. sapiens</i>	151
188573	5	22712	gtcttgaaagatggacccta	79	<i>H. sapiens</i>	152
188574	5	25543	gtttagattctatctggaga	80	<i>H. sapiens</i>	153
188575	5	29755	aaagtaccagaatatattgga	81	<i>H. sapiens</i>	154

Compositions/Formulations

Antisense compounds useful in the methods of the present disclosure may be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, resulting in, for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption.

Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921, 5,354,844, 5,416,016, 5,459,127, 5,521,291, 5,543,158, 5,547,932, 5,583,020, 5,591,721, 4,426,330, 4,534,899, 5,013,556, 5,108,921, 5,213,804, 5,227,170, 5,264,221, 5,356,633, 5,395,619, 5,416,016, 5,417,978, 5,462,854, 5,469,854, 5,512,295, 5,527,528, 5,534,259, 5,543,152, 5,556,948, 5,580,575, and 5,595,756.

The antisense compounds may be administered in a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to molecular entities that do not produce an allergic, toxic or otherwise adverse reaction when administered to a subject, particularly a mammal, and more particularly a human. The pharmaceutically acceptable carrier may be solid or liquid. Useful examples of pharmaceutically acceptable carriers include, but are not limited to, diluents, solvents, surfactants, excipients, suspending agents, buffering agents, lubricating agents, adjuvants, vehicles, emulsifiers, absorbants, dispersion media, coatings, stabilizers, protective colloids, adhesives, thickeners, thixotropic agents, penetration agents, sequestering agents, isotonic and absorption delaying agents that do not affect the activity of the active agents of the disclosure.

The antisense compounds may be pharmaceutically acceptable salts, esters, or salts of the esters, or any other compounds which, upon administration are capable of providing (directly or indirectly) the biologically active metabolite.

The term "pharmaceutically acceptable salts" as used herein refers to physiologically and pharmaceutically acceptable salts of the antisense compounds that retain the desired biological activities of the parent compounds and do not impart undesired toxicological effects upon administration. Preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860.

The antisense compounds may be prodrugs or pharmaceutically acceptable salts of the prodrugs, or other bioequivalents.

The term "prodrugs" as used herein refers to therapeutic agents that are prepared in an inactive form that is converted to an active form (i.e., drug) upon administration by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug forms of the antisense compounds are prepared as SATE [(S acetyl-2-thioethyl) phosphate]derivatives according to the methods disclosed in WO 93/24510, WO 94/26764 and U.S. Pat. No. 5,770,713.

Formulations of the growth hormone (GH) variants for therapeutic administration are prepared for storage by mixing a GH variant having the desired degree of purity with an optional pharmaceutically acceptable carrier, excipient, or stabilizer (Remington's Pharmaceutical Sciences, 16th edition, A. Oslo, A (editor) (1980)) in the form of a lyophilized cake or an aqueous solution. Parenteral formulations can be prepared by mixing the GH variant in a unit dosage injectable form (solution, suspension, or emulsion) with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are non-toxic to recipients at the dosages and

concentrations employed and are compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds known to be deleterious to polypeptides. Suitable carriers include buffers containing phosphate, borate, HEPES, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates, including glucose, mannose, or dextrans; chelating agents such as EDTA; divalent metal ions such as zinc, cobalt, or copper; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronic, or polyethylene glycol (PEG).

Additionally, the GH formulation set forth in WO 89/09614 can be employed, wherein the GH variant is contained in a composition comprising glycine, mannitol and a buffer, such as a phosphate buffer. An exemplary version of this formulation is: 0.68 g/L glycine, 18.0 g/L mannitol, 5 mM sodium phosphate, pH 7.4. Alternatively, the GH variant can be contained in a liquid formulation that does not necessarily contain mannitol or glycine and comprises 0.1 to 5% (w/v) of a non-ionic surfactant, such as polysorbate, or a poloxamer. An exemplary version of this formulation is: 5 mg/ml GH variant, 8.77 mg/ml NaCl, 2.5 mg/ml phenol, 2.0 mg/ml polysorbate 20, and 10 mM sodium citrate, pH 6.0.

The GH variant is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, for example, films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., 1983), poly(2-hydroxyethyl methacrylate) (Langer et al., 1981; Langer, 1982), ethylene vinyl acetate (Langer et al., 1982) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release GH variant compositions also include liposomally entrapped GH variants. Liposomes containing GH variants are prepared by methods known in the art (see, DE 3,218,121; Epstein et al., 1985; Hwang et al., 1980; JP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; JP 83-118008; U.S. Pat. No. 4,485,045; U.S. Pat. No. 4,544,545; and EP 102,324). Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal GH variant therapy.

The GH variant can also be formulated for local administration. Suitable formulations vary depending on the site of administration and do not differ from those known in the art. For example, GH can be formulated in a balanced salt solution for administration to the eye.

The GH variant formulation for therapeutic administration is sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic GH variant compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. GH variants ordinarily are stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 5-ml vials are

filled with 2 ml of sterile-filtered 0.5% (w/v) aqueous GH variant solution, and the resulting mixture is lyophilized.

The infusion solution is prepared by reconstituting the lyophilized GH variant using bacteriostatic water-for-injection and the like.

The formulation of pegylated GH variants is carried out as described above for GH variants generally.

Administration

The methods of the present disclosure rely on the unsuspected synergy of combining a growth hormone (GH) variant having antagonistic activity with an oligonucleotide targeted to growth hormone receptor (GHR) to reduce insulin-like growth factor I (IGF-I) levels in a subject.

In a particular embodiment of the present disclosure, the GH variant and the oligonucleotide are administered concomitantly. The GH variant and the oligonucleotide may be administered in the form of a composition comprising an admixture of both components. Alternatively, the GH variant and the oligonucleotide may be administered in separate compositions.

In one embodiment, the antisense oligonucleotide is administered systemically. As used herein "systemic administration" is a route of administration that is either enteral or parenteral.

As used herein "enteral" refers to any form of administration that involves any part of the gastrointestinal tract and includes oral administration of, for example, the antisense oligonucleotide in tablet, capsule or drop form; gastric feeding tube, duodenal feeding tube, or gastrostomy; and rectal administration of, for example, the antisense compound in suppository or enema form.

As used herein "parenteral" includes administration by injection or infusion. Examples include, intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), subcutaneous (under the skin), intraosseous infusion (into the bone marrow), intradermal, (into the skin itself), intrathecal (into the spinal canal), intraperitoneal (infusion or injection into the peritoneum), intravesical (infusion into the urinary bladder), transdermal (diffusion through the intact skin), transmucosal (diffusion through a mucous membrane), inhalational.

The antisense oligonucleotide may be administered as single dose or as repeated doses on a periodic basis, for example, daily, once every two days, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or fourteen days, once weekly, twice weekly, three times weekly, or every two weeks, every three weeks, or every four weeks.

The antisense oligonucleotide to be used in the therapy is formulated and dosed in a fashion consistent with good medical practice, taking into account the specific condition being treated, the clinical condition of the individual patient, the site of delivery of the oligonucleotide, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of oligonucleotide for purposes herein is thus determined by such considerations. The term "effective amount" in this context refers to any dose of the antisense oligonucleotide sufficient to inhibit GHR expression, under the conditions of administration.

By way of example, a dose of 25-3400, more preferably 50-1600 mg oligonucleotide may be administered to a subject. A dose of 150-400 mg, for example, a dose of 250 mg is particularly contemplated for humans. In one embodiment, a dose of 250 mg per day is administered six times over 3 weeks, on days 1, 3, 5, 7, 14 and 21. In another embodiment, a dose of 250 mg is administered once weekly, or once a fortnight.

The GH variant may be administered by, for example, continuous infusion (using, for example, minipumps such as osmotic pumps), or by injection using, for example, intravenous or subcutaneous means. In one embodiment, the GH variant is administered subcutaneously. The administration can also be as a single bolus or by slow-release depot formulation.

The GH variant composition to be used in the therapy is formulated and dosed in a fashion consistent with good medical practice, taking into account the specific condition being treated, the clinical condition of the individual patient, the site of delivery of the GH variant composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of GH variant for purposes herein (including an antagonist effective amount to counteract, for example, acromegaly) is thus determined by such considerations. The term "effective amount" in this context refers to any dose of the GH variant sufficient to antagonize GH binding, under the conditions of administration.

As a general proposition, the total pharmaceutically effective amount of the GH variant administered parenterally per dose is in the range of about 1 µg/kg/day to about 100 mg/kg/day of patient body weight, although, as noted above, this is subject to therapeutic discretion. Usually, this dose is between about 0.01 and about 10 mg/kg/day, and more usually for humans between about 0.01 and about 1 µg/kg/day. If given continuously, the GH variant is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by one to four injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution can also be employed. The key factor in selecting an appropriate dose is the result obtained, as measured for antagonists, for example, by reduction in serum GH, serum insulin-like growth factor I (IGF-I), and tumor growth, etc.

In general, a pegylated GH variant can be administered by any of the routes of administration described above. However, it is presently believed that a pegylated GH variant need not be administered as frequently as a non-pegylated GH variant. Non-pegylated GH and GH variants are typically administered at least three times a week and often daily. The pegylated forms of these proteins can be administered between about once every three days to about once a month, or more typically between about once every 6-7 days to once every two weeks. However, the pegylated GH variant Somavert is typically administered daily at doses ranging from 5 to 80 mg or more typically 10 to 30 mg per day after a loading dose of 40 mg on the first day. Somavert 30 mg/day is the highest approved daily dose regimen for acromegaly. Higher daily doses are desired in some acromegaly patients and in cancer.

The GH variant may be administered as single dose or as repeated doses on a periodic basis, for example, daily, once every two days, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or fourteen days, once weekly, twice weekly, three times weekly, or every two weeks, every three weeks, or every four weeks.

In one embodiment of the present disclosure, the GH variant is Somavert and the oligonucleotide is ATL1103 (SEQ ID NO:19) and the compounds are administered sequentially. In one embodiment, the ATL1103 oligonucleotide is first administered at a dose of 250 mg/day on days 1, 3, 5, 7, 14 and 21, and once weekly thereafter (for 5 to 12 weeks) and the GH variant Somavert is subsequently administered on the same days at 30 mg/day. Alternatively, the ATL1103 oligonucleotide may be administered once or

twice weekly (for 8 to 12 weeks) at doses of 250 mg together with once or twice or three weekly Somavert doses of 30 mg.

After 5 to 12 weeks, treatment may be continued (cycle 2) with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and with the same or increased or lower dosing frequency to achieve the desired target IGF-I levels. Post 5 to 12 weeks, the Somavert dose modification may be in about 5 to 10 mg increments and monitored at about 1 to 4 weeks to assess IGF-I levels. Post 5 to 12 weeks, the ATL1103 dose modification may be in about 25 or 50 mg increments and monitored at about 1 to 8 weeks to assess IGF-I levels. Cycle 2 can be continued if the target IGF-I normalization is achieved or a new cycle started to further optimize dosing to achieve IGF-I normalization on a patient by patient basis.

In another embodiment, a repeat 21 day treatment cycle may be used, for cancer or retinopathy treatment. The ATL1103 oligonucleotide is first administered at a dose of 250 mg/day on days 1, 3, 5, 7, 14 and 21, and the GH variant Somavert is subsequently administered on the same days at 30 mg/day. Alternatively, in a repeat cycle, the ATL1103 oligonucleotide dosing may be once or twice weekly 250 mg together with once or twice weekly Somavert doses of 30 mg on the same days or once weekly 80 mg Somavert on the same day. Alternatively, treatments may be on different days.

After 5 to 12 weeks, treatment may be continued (cycle 2) with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered the same or increased or lower dosing frequency to achieve the desired target IGF-I levels and treatment outcomes in cancer or retinopathy. Post 5 to 12 weeks, the Somavert dose modification may be in about 5 to 10 mg increments and monitored at about 1 to 4 weeks to assess IGF-I levels. Post 5 to 12 weeks, the ATL1103 dose modification may be in about 25 or 50 mg increments and monitored at about 1 to 8 weeks to assess IGF-I levels. Cycle 2 can be continued if the target IGF-I normalization is achieved or a new cycle started to further optimize dosing to achieve IGF-I normalization on a patient by patient basis.

In another embodiment, the ATL1103 drug may be dosed once or twice weekly at doses of 100, 200, 250, 300, 350, or 400 mg/day and Somavert may be dosed daily, every other day, or once or twice weekly at 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70 or 80 mg/day.

In another embodiment, the ATL1103 oligonucleotide is first administered at one of the above doses once every other week and the GH variant Somavert is subsequently administered on alternative weeks, so that the patient is on a once weekly alternative dosing regimen, first of ATL1103 and then of Somavert. In a similar embodiment, the patient is on a twice weekly dosing regimen, first of ATL1103 and then of Somavert, a few days apart, or one day apart. Somavert may also be given first and then ATL1103 dosing may follow. Somavert and ATL1103 may also be combined in a mixture and given on the same day. For example, the ATL1103 in a solution in a pre-filled syringe, may be added to the lyophilized Somavert, the Somavert reconstituted into solution, and the ATL1103 and Somavert mixture administered to the subject.

Phase I Trial of the GHR Targeting Drug ATL1103

The primary objective of the Phase I trial was to assess the safety, tolerability and pharmacokinetics (pK) of ATL1103.

The Phase I trial was a randomized, placebo controlled, double blind study of single ascending doses and multiple doses of ATL1103 in healthy adult male subjects aged between 18 and 45 years. In the single ascending dose stage of the trial, 24 subjects were administered four dose levels of ATL1103 as a single injection starting at 25 mg and escalating to 75, 250 and 400 mg or placebo. The multiple dose stage was undertaken in 12 subjects, 8 who were to receive six subcutaneous doses of 250 mg of ATL1103 and 4 subjects who received placebo administered on days 1, 3, 5, 7, 14 and 21. Subjects were monitored out to day 35.

Importantly, no serious adverse events were reported in this trial. Two subjects in the multiple dose arm withdrew from the study for reasons not related to safety. All adverse events were reported as "mild to moderate". Injection site reactions represented the majority of all the adverse events reported in the trial. There was one elevation in the liver enzyme ALT reported as an adverse event in the multiple dose stage. Importantly, the ALT levels in this subject returned to normal during the dosing phase, suggesting no residual or cumulative effect of the drug on this safety parameter.

A secondary objective of this study was to obtain data on the pharmacodynamic effects of ATL1103 on the IGF-I levels in the blood of the trial subjects. Reduction of increased levels of serum IGF-I to normal is the therapeutic endpoint in the treatment of the growth disorder acromegaly, and reducing the effects of IGF-I has a potential role in the treatment of diabetic retinopathy, nephropathy and certain forms of cancer.

As defined in the statistical analysis plan, the effect of ATL1103 on serum IGF-I was assessed as a change in IGF-I levels versus baseline (starting point) readings for those subjects who received treatment (ATL1103). Pre-dose baseline levels of IGF-I were recorded prior to the commencement of dosing and then measured at weekly intervals until the end of the monitoring period. This treated group showed a trend in reduction in IGF-I levels from day 14 to day 28, with a significant effect ($p=0.034$ one sided t-test) at day 21 with a 7% reduction in mean IGF-I levels versus baseline.

Other exploratory objectives of the study investigated the drug's mechanism of action and broader pharmacological profile, including the pharmacodynamic effects on levels of growth hormone binding protein (GHBP), insulin-like growth factor binding protein 3 (IGFBP-3), acid labile subunit of the insulin-like growth factor binding protein complex (ALS), and growth hormone (GH), as well as in vitro mitogenic and apoptotic parameters.

Notably, ATL1103 had a significant effect on reducing GHBP by 16% ($p=0.007$) at day 21 and 19% ($p<0.05$) at day 28, one week past the last dose. As circulating GHBP is produced by cleavage from the GHR, the reduction of circulating GHBP levels suggests that GHR expression is being reduced. ATL1103 also significantly reduced IGFBP-3 and ALS, both consistent with its effect on IGF-I and the fact that they are regulated by GH. There was no effect on GH levels. Specific trial details and outcomes are summarized in Tables 3-6.

TABLE 3

Summary of ATL1103 Phase I clinical trial	
Title	A randomised, placebo-controlled, double-blind, single ascending dose and multiple dose study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of subcutaneous doses of ATL1103 in healthy adult male subjects
Trial description	Phase I trial of subcutaneous administration of ATL1103 in healthy males
Objectives	Primary objectives: To assess the safety and tolerability of single subcutaneous doses (Stage A) and multiple subcutaneous doses (Stage B) of ATL1103 in healthy male subjects. To determine the single dose and multiple dose pharmacokinetic (PK) profiles of ATL1103 by the subcutaneous route of dosing Secondary objective: To assess the pharmacodynamic (PD) effects on IGF-I levels following subcutaneous administration of ATL1103 Exploratory objective: To assess the PD effects on circulating levels of (i) growth hormone (GH), (ii) insulin-like growth factor binding protein 3 (IGF-BP3), (iii) insulin-like growth factor acid-labile subunit (ALS), (iv) insulin-like growth factor II (IGF-II) and (v) growth hormone binding protein (GHBP) and on (vi) mitogenic and apoptotic activity, following subcutaneous administration of ATL1103
Main selection criteria; number of subjects	Males 18-45 years of age, BMI: 19 to 30 kg/m², healthy (determined by medical and drug history, physical examination and ECG). IGF-I levels in the normal range Stage A: 24 subjects (four groups of six subjects): randomized for 4 on ATL1103, 2 placebo Stage B: 12 subjects (one group): randomized for 8 active, 4 placebo
Test Drug, Dose and Mode of administration	Subcutaneous administration of ATL1103 in the following doses: Stage A (single dose): 0 mg (placebo); 25, 75, 250, 400 mg. Stage B (multiple doses): 0 mg (placebo); 250 mg. Six doses administered over 3 weeks (on days 1, 3, 5, 7, 14, 21).
Criteria for assessment	Safety and tolerability: Physical examinations, vital signs, adverse event monitoring and ECGs. Blood sampling for clinical safety (haematology, biochemistry), coagulation (PT, APTT and TT), urinalysis and complement assessments (Bb) Pharmacokinetic: Blood sampling for plasma ATL1103 levels at various time points over 7 days (Stage A) and 35 days (Stage B) Pharmacodynamic: IGF-I: Serum samples collected at least weekly to day 35. Exploratory PD: Blood sampling for GH, and the following for Stage B only: IGF-BP3, ALS, IGF-II, GHBP and in vitro mitogenic and apoptotic parameters on Days 1, 7, 21 and 28 of the study
Subject withdrawals	Two subjects withdrew from the study after the fifth dose of ATL1103 due to 1) withdrawal of consent 2) subject lost to follow up. No subject withdrew or was withdrawn for safety reasons.
Outcomes	Primary objective outcomes ATL1103 was considered safe and generally well tolerated at the doses used in the study There were no serious adverse events reported There were 24 treatment-emergent adverse events (TEAE) in Stage A (19 in the 16 ATL1103-treated subjects, 5 in the 8 placebo-treated subjects), all reported as mild or moderate. In the ATL1103-treated subjects most common adverse events reported were pain at injection site (6), headache (5), influenza-like illness (2). There were 25 TEAEs in Stage B (18 in the 8 ATL1103-treated subjects; 7 in the 4 placebo-treated subjects). All were reported as mild. In ATL1103-treated subjects the most commonly reported adverse events were injection site reactions (13). Notably, although influenza-like illness (inc muscle aching and fever) was seen in two subjects after single doses of 400 mg ATL1103, this was not seen after repeated doses of 250 mg. Increased ALT levels were reported for one subject in Stage B at Day 11. ALT levels returned to the normal range by pre-dose day 21 and remained within normal range throughout the rest of the study period. A summary of the pharmacokinetic parameters is shown in Table 1. Secondary & Exploratory objectives outcomes The effect of ATL1103 on serum IGF-I levels and on the exploratory PD markers were determined as change from baseline levels. For IGF-I there was a clear trend for mean levels to be lower than baseline on days D14, 21, 28, 35 of the study with a statistically significant effect reached by day 21 (Table 2) No treatment-related effects were apparent in growth hormone levels (data not shown) The inhibitory effect of ATL1103 on other exploratory PD markers is shown in Table 3. Of particular note is the ATL1103-related inhibition of circulating GHBP. GHBP is produced by cleavage from the GHr receptor, so reduction of circulating GHBP levels suggests that GHr expression is reduced. This provides support for ATL1103 working via an antisense mechanism of action. IGF-BP3 and ALS reductions are consistent with the effect of ATL1103 on IGF-I (Table 3).

TABLE 4

Summary of pharmacokinetic parameters (mean ± SD)				
	n	C _{max} (ng/mL)	T _{max} (hr)	AUC _{last} (hr * ng/mL)
Single Dose				
25 mg	4	466 ± 136	3.25 ± 0.5	3711 ± 822
75 mg	4	3139 ± 1576	3.25 ± 0.96	21342 ± 4755
250 mg	4	12383 ± 3000	2.5 ± 1.29	88942 ± 11295

TABLE 4-continued

Summary of pharmacokinetic parameters (mean ± SD)				
	n	C _{max} (ng/mL)	T _{max} (hr)	AUC _{last} (hr * ng/mL)
60				
400 mg	4	14343 ± 2823	3.25 ± 0.96	151123 ± 29434
Multiple dose (250 mg; Pharmacokinetic population)				
Day 1	6	8318 ± 2623	3.84 ± 1.49	58400 ± 11220
Day 21	6	8557 ± 1431	3.00 ± 0.63	91870 ± 16795

TABLE 5

Serum IGF-I levels in ATL1103-treated subjects in Stage B		
Pre-dose IGF-I: 36.57 ± 13.16 nmol/L; mean ± SD		
Study day	Change from pre-dose Mean ± SD (% change from pre-dose)	Probability < (-t) ^a
Day 3	+2.65 ± 9.47 (12%)	>0.500
Day 5	+0.32 ± 7.42 (6%)	>0.500
Day 7	+0.77 ± 7.51 (6%)	>0.500
Day 14	-1.43 ± 7.19 (-2%)	0.323
Day 21	-3.40 ± 3.59 (-7%)	0.034
Day 28	-3.43 ± 7.53 (-7%)	0.157
Day 35	-1.9 ± 5.08 (-1%)	0.201

^a1-sided t-test on change from pre-dose at each time point; alternate hypothesis is suppression Per protocol population; (n = 6 at each time point)

TABLE 6

Exploratory PD assessments of ATL1103 subjects in Stage B				
Pre-dose value/Mean change from pre-dose (% change from pre-dose)				
	GHBP (pmol/L)	IGF-BP3 (ng/ml)	IGF-II (ng/ml)	ALS (mU/ml)
Pre-dose	1053.8 ± 664	2906.2 ± 320.8	722.3 ± 113.8	1710.2 ± 305.4
Day 7	-31.2 (0%)	-310.5** (-10%)	-101.8*** (-14%)	-115.3 (-5%)
Day 21	-169.5** (-16%)	-228.3* (-8%)	-57.5 (-8%)	-160.7 (-9%)
Day 28	-233.3* (-19%)	-160.5 (-5%)	-29.2 (-5%)	-188.7* (-10%)

*P < 0.050,

**P < 0.010,

***P < 0.001;

1-sided t-test on change from pre-dose at each time point; alternate hypothesis is suppression Per protocol population; (n = 6 at each time point)

Example 2

Co-Administration of an Antisense Oligonucleotide and Somavert Including in Subjects in Need of Serum IGF-I Reduction

ATL1103 is to be subcutaneously administered at 250 mg per day, six times over 3 weeks on days 1, 3, 5, 7, 14 and 21.

Somavert will be dosed subcutaneously at 20 mg per day for 7 days starting on day 1 or day 24.

Although not wishing to be limited to theory, ATL1103 is cleared from the blood after each dose and accumulates in the liver and other organs because of its long tissue half life. ATL1103 will be working to decrease GHR protein on the cell surface of hepatocytes and other liver cells and other organs and decrease the resultant soluble form of GHR in the blood (GHBP) which is cleaved from the cell surface GHR protein.

Control groups: Somavert is to be administered alone for a similar 7 or 24 day period or ATL1103 is to be administered for a similar 21 day period to normal volunteers or subjects in need of reduction of serum IGF-I.

Pharmacodynamic and pharmacological effects will be assessed as described in Example 1 with similar assays and additional assays where useful, for example, for serum IGF-I and GH.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I levels, and optionally, GH levels.

Example 3

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Acromegaly

Groups of 15 acromegalics are to be dosed with ATL1103 as described in Example 1 and with additional once weekly dosing for 6 weeks and either (i) 30 mg once weekly Somavert or (ii) 80 mg once weekly Somavert for 6 weeks, starting on day 24.

Control groups: ATL1103 or Somavert is to be administered alone for a similar 9 week period or 6 week period, respectively, to acromegalic patients. It has previously been shown that serum IGF-I was reduced by 16% and 31% and 12.5% and 26.7% of patients were normalized for serum

IGF-I levels when administered Somavert alone at 30 and 80 mg, respectively for 6 weeks at these doses. Somavert once weekly dosing was abandoned for daily dosing for acromegalics to normalize the serum IGF-I of a greater number of acromegalics.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I levels.

Example 4

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Acromegaly

Groups of 15 acromegalics are to be dosed with 200 mg ATL1103 once weekly dosing for 13 weeks and either (i) 30 mg once weekly Somavert or (ii) 80 mg once weekly Somavert for 13 weeks on the same days as ATL1103.

Control groups: ATL1103 or Somavert is to be administered alone for a similar 13 week period to acromegalic patients.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I levels.

Example 5

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Acromegaly

Groups of 15 acromegalics are to be dosed with 200 mg ATL1103 once or twice weekly dosing for 13 weeks and

47

either (i) 30 mg once weekly Somavert or (ii) 30 mg twice weekly Somavert for 13 weeks on the same days as ATL1103.

Control groups: ATL1103 or Somavert is to be administered alone for a similar 13 week period to acromegalic patients.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I levels.

Example 6

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Diabetic Retinopathy

Groups of 15 patients with diabetic retinopathy are to be dosed with 200 mg ATL1103 once or twice weekly dosing for 13 weeks and either (i) 30 mg once weekly Somavert or (ii) 30 mg twice weekly Somavert for 13 weeks on the same days as ATL1103.

Control groups: ATL1103 or Somavert is to be administered alone for a similar 13 week period to diabetic retinopathy patients.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target IGF-I levels, and optionally GH levels and outcomes in retinal disease.

Example 7

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Cancer

Groups of 15 patients with cancer associated with increased IGF-I are to be dosed with 200 mg ATL1103 once or twice weekly dosing for 13 weeks and either (i) 30 mg once weekly Somavert or (ii) 30 mg twice weekly Somavert for 13 weeks or (iii) 80 mg once weekly or (iv) 80 mg twice weekly Somavert.

Control groups: ATL1103 or Somavert is to be administered alone, with patients standard medication, for a similar 13 week period to cancer patients.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target IGF-I levels, and optionally GH levels and outcomes in cancer.

Example 8

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Acromegaly

Somavert will be dosed subcutaneously at the doses acromegaly patients are currently using for their treatment, for example, 10, 15, 20, 25, 30, 35, 40, 45, 50 mg/day or more.

ATL1103 is to be subcutaneously administered at, 250 mg per day, six times over 3 weeks on days 1, 3, 5, 7, 14 and 21.

48

Pharmacodynamic and pharmacological effects will be assessed as described in Example 1 with similar assays and additional assays where useful, for example, for serum IGF-I and GH.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I and, optionally, GH levels.

Example 9

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Acromegaly

Somavert will be dosed subcutaneously at the doses acromegaly patients are currently using for their treatment, for example, 10, 15, 20, 25, 30, 35, 40, 45, 50 mg/day or more.

ATL1103 is to be subcutaneously administered at 100, 150, 200, 250, 300, 350 or 400 mg over 3 weeks once or twice weekly or until a cumulative dose of ~1200-1800 mg.

Pharmacodynamic and pharmacological effects will be assessed as described in Example 1 with similar assays and additional assays where useful, for example, for serum IGF-I and GH.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I and, optionally, GH levels.

Example 10

Co-Administration of an Antisense Oligonucleotide and Somavert for the Treatment of Acromegaly

Somavert will be dosed subcutaneously at the doses acromegaly patients are currently using for their treatment, for example, 10, 15, 20, 25, 30, 35, 40, 45, 50 mg/day or more.

ATL1103 is to be subcutaneously administered at 100, 150, 200, 250, 300, 350 or 400 mg over 4 weeks once or twice weekly, or over 6 weeks once or twice weekly, or over 8 weeks once or twice weekly, or over 12 weeks once or twice weekly.

Pharmacodynamic and pharmacological effects will be assessed as described in Example 1 with similar assays and additional assays where useful, for example, for serum IGF-I and GH.

Treatment is to be continued with the same or increased or lower doses of Somavert, and with the same or increased or lower doses of ATL1103, and administered with the same or increased or lower dosing frequency to achieve the desired target serum IGF-I and, optionally, GH levels.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

All publications discussed and/or referenced herein are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission

that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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<400> SEQUENCE: 51

actttttggc atctgcctca 20

<210> SEQ ID NO 52
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<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 52

gatgcacttt ttggcatctg 20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:

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<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 53

cagtcgcatt gagtatgagg                                20

<210> SEQ ID NO 54
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

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ctctttgtca ggcaagggca                                20

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<223> OTHER INFORMATION: oligonucleotide

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gtgctcacat agccacatga                                20

<210> SEQ ID NO 56
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<212> TYPE: DNA
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<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 56

aagaaaggct aaggcatgat                                20

<210> SEQ ID NO 57
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<212> TYPE: DNA
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<220> FEATURE:
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aaatacgtag ctcttgggaa                                20

<210> SEQ ID NO 58
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<400> SEQUENCE: 58

caatcactgc tactaaacag                                20

<210> SEQ ID NO 59
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aaacatagcc attcaatgct                                20

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<210> SEQ ID NO 60
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gtgctatggt ttgcattcaa 20

<210> SEQ ID NO 61
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gttttacata tccaaactat 20

<210> SEQ ID NO 62
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catcaaccaa gatttggtga 20

<210> SEQ ID NO 63
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gaggctatag atcttatctc 20

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tagtgagaaa gaaagtttct 20

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aatgctctca agaatgatgt 20

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<400> SEQUENCE: 66

acactcaatt ctagcttttc

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catctattac aaataacatg

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<212> TYPE: DNA

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<223> OTHER INFORMATION: oligonucleotide

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tctacactga tgatacttta

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<212> TYPE: DNA

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<223> OTHER INFORMATION: oligonucleotide

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cacagctttg aattgaatta

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<210> SEQ ID NO 71

<211> LENGTH: 20

<212> TYPE: DNA

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<223> OTHER INFORMATION: oligonucleotide

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aggctgttgt gaaatagtaa

20

<210> SEQ ID NO 73

<211> LENGTH: 20

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atagaaatgt tgtcaggctg 20

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ccaaaatgac attctgagac 20

<210> SEQ ID NO 75
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ataatggctt atgtggccac 20

<210> SEQ ID NO 76
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<400> SEQUENCE: 76

agttatgtga ccttgattga 20

<210> SEQ ID NO 77
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<212> TYPE: DNA
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<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 77

ttgagtgttc ctaaaatgaa 20

<210> SEQ ID NO 78
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<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 78

atggaggctg gaggttcaaa 20

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tagggtccat ctttcaagac 20

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<400> SEQUENCE: 80

tctccagata gaatctaaac 20

<210> SEQ ID NO 81
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 81

tcctcaatatt ctggtacttt 20

<210> SEQ ID NO 82
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 82

tattagttac cttgaggaga 20

<210> SEQ ID NO 83
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<212> TYPE: DNA
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<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 83

attttccttc ctagaaaata 20

<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 84

gaatggaaag aatgccctga 20

<210> SEQ ID NO 85
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<212> TYPE: DNA
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<400> SEQUENCE: 85

gaaagaatgc cctgattatg 20

<210> SEQ ID NO 86
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 86
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<210> SEQ ID NO 87
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<400> SEQUENCE: 87
attgagctag atattgatga                20

<210> SEQ ID NO 88
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<212> TYPE: DNA
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<400> SEQUENCE: 88
gacacagaca gacttctaag                20

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 89
agcgacatta caccagcagg                20

<210> SEQ ID NO 90
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 90
aaccaagagg acatttacat                20

<210> SEQ ID NO 91
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 91
agaggacatt tacatcacca                20

<210> SEQ ID NO 92
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 92
acatttacat caccacagaa                20

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<210> SEQ ID NO 93
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 93

tacatcacca cagaaagcct 20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 94

caccacagaa agccttacca 20

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 95

tatgtgagca cagaccaact 20

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 96

gagcacagac caactgaaca 20

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 97

ccaactgaac aaaatcatgc 20

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 98

tctgctactt tgctgctatg 20

<210> SEQ ID NO 99
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: target sequence

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<400> SEQUENCE: 99

tttctatagc caaaaatagc 20

<210> SEQ ID NO 100

<211> LENGTH: 20

<212> TYPE: DNA

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aatagctaaa tacctcaatc 20

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 102

ctacaggtat ggatctctgg 20

<210> SEQ ID NO 103

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 103

cacagcagct atccttagca 20

<210> SEQ ID NO 104

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 104

taatccaggc ctaaagacaa 20

<210> SEQ ID NO 105

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 105

tctaaggagc ctaaattcac 20

<210> SEQ ID NO 106

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 106

gaacctagga cccatacagc 20

<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 107

gctggggaaa acagctgtta 20

<210> SEQ ID NO 108
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 108

tggtggtaca gtggatgaaa 20

<210> SEQ ID NO 109
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 109

ctgttgatga aatagtgcaa 20

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 110

tagtgcaacc agatccacc 20

<210> SEQ ID NO 111
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 111

gatgggaagc accacgcaat 20

<210> SEQ ID NO 112
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<212> TYPE: DNA
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<400> SEQUENCE: 112

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atggaaaatg atggacccta	20
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 119

acagtgatga ctcttggggt                20

<210> SEQ ID NO 120
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 120

gctagatatt gatgagccag                20

<210> SEQ ID NO 121

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<210> SEQ ID NO 122
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 122

atttcaatgc caatgacata                20

<210> SEQ ID NO 123
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 123

aagcagatct cttatgcctt                20

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 124

tcctactgaa ggagctgagt                20

<210> SEQ ID NO 125
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 125

agaataaggc agggatgtcc                20

<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 126

acttccttat ggacaatgcc 20

<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 127

tgaggcagat gccaaaaagt 20

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 128

cagatgccaa aaagtgcac 20

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 129

cctcactc aatgcgactg 20

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 130

tgcccttgcc tgacaaagag 20

<210> SEQ ID NO 131
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 131

tcatgtggct atgtgagcac 20

<210> SEQ ID NO 132

<400> SEQUENCE: 132

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<210> SEQ ID NO 133
<211> LENGTH: 20
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 133

ttccaagag ctacgtattt                20

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<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 134

ctgttttagta gcagtgattg                20

<210> SEQ ID NO 135
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 135

ttgaatgcaa accatagcac                20

<210> SEQ ID NO 136
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 136

atagtttgga tatgtaaac                20

<210> SEQ ID NO 137
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 137

tcaccaaadc ttggttgatg                20

<210> SEQ ID NO 138
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 138

gagataagat ctatagcctc                20

<210> SEQ ID NO 139
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 139

agaaactttc tttctcacta                20

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<210> SEQ ID NO 140
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 140

acatcattct tgagagcatt 20

<210> SEQ ID NO 141
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 141

gaaaagctag aattgagtgt 20

<210> SEQ ID NO 142
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 142

gctatggttt tctccaagag 20

<210> SEQ ID NO 143
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 143

taaagtatca tcagtgtaga 20

<210> SEQ ID NO 144
<211> LENGTH: 20
<212> TYPE: DNA
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taattcaatt caaagctgtg 20

<210> SEQ ID NO 145
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 145

agctgtgtgt ttggaagact 20

<210> SEQ ID NO 146
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<212> TYPE: DNA
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<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 146

ttactatttc acaacagcct                                20

<210> SEQ ID NO 147
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: target sequence

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cagcctgaca acatttctat                                20

<210> SEQ ID NO 148
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<212> TYPE: DNA
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<220> FEATURE:
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gtctcagaat gtcattttgg                                20

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<212> TYPE: DNA
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gtggccacat aagccattat                                20

<210> SEQ ID NO 150
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tcaatcaggg tcacataact                                20

<210> SEQ ID NO 151
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 151

tttgaacctc cagcctccat                                20

<210> SEQ ID NO 152
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 152

gtcttgaaag atggacccta                                20

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<210> SEQ ID NO 153
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: target sequence

<400> SEQUENCE: 153

gtttagattc tatctggaga

20

<210> SEQ ID NO 154
 <211> LENGTH: 20
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aaagtaccag aatatttga

20

The invention claimed is:

1. A method of reducing the level of insulin-like growth factor I (IGF-I) in a subject, the method comprising administering a growth hormone (GH) variant having GH antagonistic activity and comprising the following amino acid substitutions: H18D, H21N, G120K, R167N, K168A, D171S, K172R, E174S, I179T compared with the native GH amino acid sequence shown in SEQ ID NO:2, in combination with an oligonucleotide 15 to 30 nucleobases in length comprising at least one modified internucleoside linkage, sugar moiety, or nucleobase, targeted to a nucleic acid encoding human growth hormone receptor (GHR) so as to inhibit expression of the GHR, thereby reducing the level of IGF-I in the subject.

2. The method of claim 1, wherein the nucleic acid is as shown in SEQ ID NO:4 or SEQ ID NO:5.

3. The method of claim 1, wherein the oligonucleotide is a DNA oligonucleotide.

4. The method of claim 1, wherein the oligonucleotide is a RNA oligonucleotide.

5. The method of claim 4, wherein the oligonucleotide is a short interfering RNA (siRNA).

6. The method of claim 1, wherein the oligonucleotide is a chimeric oligonucleotide.

7. The method of claim 1, wherein the oligonucleotide has at least 70% complementarity with the nucleic acid encoding human GHR.

8. The method of claim 1, wherein the oligonucleotide has at least 80% complementarity with the nucleic acid encoding human GHR.

9. The method of claim 1, wherein the oligonucleotide has at least 90% complementarity with the nucleic acid encoding human GHR.

10. The method of claim 1, wherein the oligonucleotide has at least 95% complementarity with the nucleic acid encoding human GHR.

11. The method claim 1, wherein the oligonucleotide comprises at least an 8 consecutive nucleobase portion of SEQ ID NO: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, or 81.

12. The method of claim 1, wherein the oligonucleotide consists of the nucleobase sequence of SEQ ID NOs: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, or 81.

13. The method of claim 12, wherein the oligonucleotide consists of the nucleobase sequence of SEQ ID NO:6.

14. The method of claim 1, wherein the oligonucleotide specifically hybridizes with a region encoding human GHR, wherein the region comprises a translation initiation codon, a termination codon, a coding region, a 5' untranslated region, a 3' untranslated region, an intron:exon junction or an exon:intron junction.

15. The method of claim 14, wherein the region comprises at least an 8 consecutive nucleobase portion of a sequence selected from SEQ ID NOs: 84-154.

16. The method of claim 1, wherein the oligonucleotide comprises at least an 8 consecutive nucleobase portion complementary to a region of SEQ ID NO:4 selected from the group consisting of nucleotides 260-339, 332-351 and 344-423 of SEQ ID NO:4.

17. The method of claim 1, wherein the oligonucleotide inhibits the expression of GHR and/or growth hormone binding protein (GHBP) by at least 15%.

18. The method of claim 1, wherein the oligonucleotide comprises at least one 2'-O-methoxyethyl sugar moiety.

19. The method of claim 1, wherein the oligonucleotide comprises at least one phosphorothioate internucleoside linkage.

20. The method of claim 1, wherein the oligonucleotide comprises at least one 5-methylcytosine.

21. The method of claim 1, wherein the oligonucleotide consists of 20 linked nucleosides, wherein the oligonucleotide consists of a nucleobase of SEQ ID NO:6; and wherein the oligonucleotide consists of a ten deoxynucleotide region flanked on both the 5' end and the 3' end of said ten deoxynucleotide region with five 2'-O-(2-methoxyethyl) nucleotides, and wherein each internucleoside linkage in the oligonucleotide is a phosphorothioate linkage, and wherein each cytosine in said oligonucleotide is a 5-methylcytosine.

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